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Shear, C. L.

# Endothia parasitica & related species

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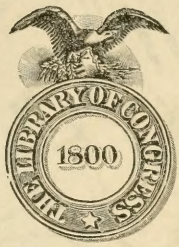


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UNITED STATES DEPARTMENT OF AGRICULTURE

BULLETIN No. 380

Contribution from the Bureau of Plant Industry
WM. A. TAYLOR, Chief

Washington, D. C. PROFESSIONAL PAPER January 15, 1917

# ENDOTHIA PARASITICA AND RELATED SPECIES

By

C. L. SHEAR, Pathologist, and NEIL E. STEVENS, Pathologist, Fruit-Disease Investigations, and RUBY J. TILLER, Scientific Assistant, Office of Investigations in Forest Pathology

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## BUREAU OF PLANT INDUSTRY.

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## TAXONOMY.

### INTRODUCTION.

The discovery of a serious canker of the chestnut in the New York Zoological Park in 1904, by Merkel (49),[2] first attracted the attention of pathologists and foresters to what has proved to be one of the most serious epidemics of a plant disease ever known in this country.

The fungus which was found associated with these cankers (Pl. I and Pl. II, fig. 1) and soon demonstrated experimentally to be their cause was described by Murrill (57) in 1906 as a new species of Diaporthe (*D. parasitica*). Search for the fungus in other places in New York and vicinity soon showed that it was already established and apparently rapidly spreading. Investigations which have been continued and extended from year to year have shown

[1] Formerly Pathologist, Office of Investigations in Forest Pathology.

[2] Serial numbers in parentheses refer to "Literature cited," at the end of the bulletin.

NOTE.—This bulletin is of value to botanists, especially plant pathologists and mycologists, and to all persons who are interested in the study of chestnut blight.

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conclusively that the disease is spreading very rapidly, especially west and south from New York and also north and east.

The exact identity and relationships of the fungus causing the disease and the origin of the epidemic soon became the subject of study by various mycologists and pathologists. Different explanations were offered for the sudden appearance and behavior of the disease, one view being that the fungus was probably a foreign parasite which had been introduced; another, that the organism was probably a native species which had recently attracted attention, chiefly by reason of the weakened condition of the chestnut trees due to abnormal climatic or other conditions.

In attacking the problem of the origin of the parasite and its possible control, it was evidently necessary to secure all the information possible in regard to its life history, identity, distribution, and relationships. The senior writer in an unpublished paper prepared in 1908 pointed out the close relationship and possible identity of *Diaporthe parasitica* with certain species of Endothia. Clinton (16) and Farlow (28) soon after also made the same suggestion. Two species of Endothia had already been described from this country by Schweinitz (74) under the old generic name, Sphaeria. These, however, had in recent years been regarded as a single species and referred to *Endothia gyrosa* (Schw.). Owing to a lack of knowledge of the types of these two species and for want of good specimens showing ascospores, it was difficult to determine what species of Endothia were indigenous in the eastern United States. Since it had been suggested that *Diaporthe parasitica* was either identical with one of Schweinitz's species or a mere variety of it, the present writers undertook a thorough study of the genus Endothia in its taxonomic, ecological, and pathological relations. It was first necessary to determine the identity of the two species already described by Schweinitz from America and also to learn their distribution and host relations. As one or both of Schweinitz's species were reported to occur in southern Europe on chestnut, it was important to obtain exact knowledge in regard to the identity and relationships of the European species. The senior writer spent several months in Europe collecting material of Endothia in the field and studying herbarium specimens of types and authentic collections of Schweinitz and other authors. Material was also acquired by collection and exchange with pathologists and mycologists in nearly every region of the world in which Endothia was known to occur. Comparative cultural studies were made of all the living material secured, as well as inoculation experiments on various hosts. The recent discovery of the typical chestnut-blight parasite, *Endothia parasitica*, by Meyer (27, 76, 78), in China and Japan and the failure to find in Europe or America any native form which would produce the disease appear to settle beyond question its foreign origin.

The present paper presents the results of several years' field and laboratory study of the species of Endothia. This includes the study of practically all the herbarium material of this genus preserved in the principal herbaria of Europe and America; also field and laboratory studies of over 600 new collections from various localities and hosts in America, Europe, and Asia. Over 4,000 cultures have been studied and about the same number of inoculations made. These studies include the systematic relations of the species of Endothia and their physiological behavior on various culture media and under various conditions of light, moisture, and temperature; also inoculation experiments with the various species on various hosts.

The writers wish to record here their grateful acknowledgment and thanks for opportunities to examine specimens and for assistance rendered by various mycologists and pathologists and directors and curators of botanical gardens and museums, especially the following: Prof. O. Comes, Naples; Prof. Romualdo Pirotta, Prof. Giuseppi Cuboni, and Drs. E. Pantanelli and L. Petri, Rome; Prof. P. Baccarini, Florence; Prof. P. A. Saccardo, Padua; Dr. G. Briosi, Pavia; Dr. J. Briquet, Delessert Herbarium, Geneva; M. G. Beauverd, Boissier Herbarium, Geneva; Prof. L. Jost, Strasburg; Prof. W. Pfeffer, Leipzig; Dr. G. Lindau, Berlin; Dr. J. W. C. Goethart, Leiden; Prof. H. O. Juel, Upsala; Dr. P. Hariot, Paris; Sir David Prain, Kew; Dr. A. B. Rendle, British Museum; Prof. I. B. Balfour, Edinburgh; Prof. T. Petch, Peredeniya, Ceylon; Dr. C. Spegazzini, La Plata, Argentina; Dr. W. G. Farlow, Harvard University; Dr. W. A. Murrill, New York Botanical Garden; Mr. Stewardson Brown, Philadelphia Academy of Science; Dr. G. T. Moore, St. Louis Botanical Garden; Prof. E. Bethel, Denver, and Drs. G. P. Clinton, P. J. Anderson, and F. D. Heald. The writers have also received specimens and cultures from numerous other colleagues which have been of great assistance and are duly appreciated.

## THE GENUS ENDOTHIA.

The genus Endothia was established by Elias Fries in 1849 (33, pp. 385–386), as follows:

(X. Endothia. Fr.*)

* Colore rubro fulvove, habitu Tuberculariae, peritheciis cellulosis difformibus pallidis, ascis diffluentibus, facile distinctum genus, nobis exoticum, sed jam in Europa australi obvium v. c. *Sph. gyrosa* Schw.—et subgenus, tuberculo uniloculari, sistit *S. Tuberculariа* Dec. Omnium horum generum characteres proxime plenius exhibeamus, examinatis multis speciebus exoticis.

The description of the genus transcribed here was published as a footnote in the work cited and was evidently based on the specimens contained in Fries's herbarium at the time the book was written.

Fries (31, p. 73) had at that time, according to his own statement, authentic specimens of *Sphaeria gyrosa* sent him by Schweinitz and also the specimens collected by Guepin and Levieux in France, which he identified as this species. In Fries's herbarium at Upsala at present are found specimens of true *S. gyrosa* Schw. with Schweinitz's autograph label, but no specimens of *S. gyrosa* could be found attributed to Guepin or Levieux. There is a small packet marked "*Sph. gyrosa*," apparently in Fries's handwriting, but there seems to have been some confusion in the labeling or mounting of this specimen, as a small stroma of *Hypoxylon annulatum* which does not look at all like Endothia is included. The other piece consists of an irregular pycnidial stroma which may be the southern European specimens referred to in the description quoted. Fries's identification of this European material as *E. gyrosa* was apparently based chiefly upon its superficial resemblance to the pycnidial stromata of Schweinitz's American specimens. The senior writer has seen and made a careful microscopic examination of a specimen collected by Guepin in France and preserved in De Notaris's herbarium at Rome. It is labeled "*Sphaeria gyrosa* Fries, Guepin, Angers." The specific name "gyrosa" has been crossed out by De Notaris and "radicalis Schw." written above it and the date "April, 1845," added. This appears to be a part of the same collection that Guepin sent to Fries, as the specimen agrees well with Fries's description and consists chiefly of pycnidial stromata which are rather larger than is usual for *Sphaeria radicalis* and show considerable superficial resemblance to the stromata of *Sphaeria gyrosa* Schw. A thorough examination of this specimen, however, reveals a few perithecia and ascospores, which leave no doubt that it is *S. radicalis* of Schweinitz, as indicated by De Notaris on the label. What the plant sent Fries by Levieux was is unknown, as no specimen so labeled could be found in Fries's herbarium. It appears from all the evidence at hand that Fries was mistaken in his identification of the material from Levieux and Guepin, as no specimens of the true *Sphaeria gyrosa* Schw. have yet been seen from Europe.

There seems to be no doubt, however, that Fries intended the true *Sphaeria gyrosa* Schw. to represent the type of his genus Endothia, as he had a part of Schweinitz's original collection at the time and never definitely placed any other species in the genus; hence, *Sphaeria gyrosa* Schw. should be adopted as the nomenclatorial type of the genus. It is clear from Fries's writings and specimens that he knew *Sphaeria radicalis* Schw., as he had American specimens from Schweinitz as well as European collections at the time he founded this genus. He did not, however, apparently regard it as congeneric with *S. gyrosa*. His specimens of *S. radicalis* show

the typical perithecia with necks, whereas no perithecia have been found in any of Schweinitz's specimens of *S. gyrosa* examined by the writers. Fries, in common with Schweinitz, regarded the pycnidial cavities of *S. gyrosa* as perithecia. When the pycnidia of *S. gyrosa* are mistaken for perithecia and compared with the real perithecia of *S. radicalis* the differences appear marked. It was therefore quite as natural for Fries to place the two species in different genera as it had been for Schweinitz to place them in different tribes of the genus Sphaeria. Fries's mistake in describing as perithecia the pycnidial cavities in the stroma of *S. gyrosa* explains his reference to the asci as "ascis diffluentibus." Believing that he had perithecia but finding no asci, he interpreted this as indicating that they had disappeared.

According to the plan of accepting only names originally applied to the ascospore stage, this name would be invalid, as proposed by Fries, and would be attributed to De Notaris, who placed the perithecial form of *Sphaeria radicalis* Schw. in the genus and described the ascospores. There is not the slightest question, however, in regard to the identity of the different stages of this fungus and their genetic connection, and the name Endothia has been almost invariably applied to these two species in both stages.

### SYNONYMY.

There are only two true generic synonyms of Endothia: Endothiella Saccardo, 1906 (71, p. 278) and Calopactis H. and P. Sydow, 1913 (81, p. 82). Endothiella was based on *Endothiella gyrosa* Sacc., which, according to authentic specimens from Saccardo, is undoubtedly the pycnidial form of *Endothia fluens* as found in Italy. Calopactis was based on *C. singularis*, the pycnidial condition of *Endothia singularis* (H. and P. Syd.) S. and S. Ascospore cultures of this have not yet produced any pycnidia, but the proof of the genetic connection of the two stages appears rather conclusive from the occurrence of pycnidia and perithecia in the same stroma, as shown in Plate XII. Perithecial stromata and ascospores were also found in the specimen of the Sydow exsiccati in the Pathological and Mycological Collections of the Bureau of Plant Industry.

Von Höhnel (43, p. 1479–1481) considers Cryphonectria Sacc. as a synonym of Endothia, taking *C. gyrosa* (B. and Br.) as the type of that genus because it is the first species listed by Saccardo in connection with his description of the genus. Saccardo, however, had previously established Cryphonectria as a subgenus, with *C. abscondita* as the type, which is not an Endothia. Valsonectria is also considered by Von Höhnel a synonym of Endothia, but apparently he had not compared specimens of Spegazzini's fungus, which is found upon examination of the type species to be separate from Endothia. The

Tulasnes (83, p. 87–89) do not appear to have regarded Endothia as distinct from Melogramma, to which they referred *E. gyrosa*. The type of Melogramma, however, is *M. melogramma* (Bull.), which has a somewhat similar stroma, but the ascospores are 3-septate and dark colored and the perithecia not separable from the stroma, while the pycnospores are long, slender, and curved.

### STUDY OF EARLY COLLECTIONS AND TYPES.

There has always been more or less uncertainty in regard to the identity of the older species of this genus of fungi. In order to get more light on this subject, a thorough study of all the available material in the way of literature, type specimens, and manuscripts was made. The first species to be described in this country was *Sphaeria gyrosa* Schw. This was collected by Schweinitz at Salem, N. C., and published in 1822 (72, p. 3).[1] Two hosts were given in the original description, Fagus and Juglans.

As Schweinitz's description was prepared before the advent of careful microscopical studies and spore measurements, it is impossible to identify the organism satisfactorily from the original description. It was, therefore, important, if possible, to locate the type specimens upon which the description was based. Schweinitz's herbarium was left at his death, in 1834, to the Philadelphia Academy of Science. His specimens of fungi at the time they were transferred to the academy were contained in small, folded paper packets, as shown in Plates V and VI. These packets were then inclosed in other heavy paper wrappers, folded to small quarto size, and three or four of these large packets, each bearing a manuscript list of the species contained, were then inclosed in quarto pasteboard covers, tied with tape. The individual species packets were labeled in Schweinitz's handwriting, with the name of the species and the locality of the collection, as shown in Plate V, figure 2.

These species packets frequently bore the names of several localities, but usually two, Salem [N. C.] and Bethlehem [Pa.], as most of his collecting was done at these places. This fact, in addition to the evidence afforded by the specimens in the packets, clearly indicates Schweinitz's method of handling his specimens.

Frequently some of the specimens in a packet show the remains of a gummed strip. This will be noticed in Plate III, which indicates

[1] 24. *Sphaeria gyrosa* Sz.

*S. subperipherica* minor gregaria subconfluens aurantio miniata, sphaerulis gyrosis farctis demum prominulis pulverulentis, stromate lutescenta.

In cortice nondum corrupto etiam vivo Fagorum et Iuglandum. Junior planiuscula, ubi adolevit sistit corpus subrotundum, tuberculis minimis et magoribus asperum et gyrosum. Sphaerulae farctae, teretes, supra gyrosae, paucae, radiatim divergentes a superficie ad centrum fere stromatis continuantur, primum sublantes, demum prominulae, cortice pulverulento; ipsum tamen centrum farinacea carne componitur. Gelatina asciphora albet. Ostiola indistincta.—Transitum facit ad Sphaerias septimae divisionis.

that at one time the specimen was apparently attached to a sheet by a gummed paper strip. This seems to have been the way in which Schweinitz originally mounted his specimens, but later, apparently, he changed to the plan of putting them in paper packets and removed those which had been attached to sheets. It is clear from an examination of the specimens still found in some of the original packets that two or more different hosts were sometimes included. In some cases as many as four or five different collections appear to have been placed in the same packet and each new locality added on the outside. This method of keeping specimens makes it rather difficult in some cases to determine which belongs to the first collection. In the case of *Sphaeria gyrosa* but two localities are indicated on the packet, Salem and New England. (See Pl. VI, fig. 2.)

The difficulties in determining the true type specimen of any species would have been sufficiently great if the collection had been preserved as it was left by Schweinitz. The matter is, however, further complicated by the later handling and rearrangement of the collection. Some time after Schweinitz's death (the exact date the writers have been unable to determine) his collection of fungi was more or less completely rearranged and mounted. The greater part of this work was evidently done by Dr. Ezra Michener. Dr. Michener was a lifelong resident of Chester County, Pa. He early became interested in botany, and in 1840 was elected a correspondent of the Philadelphia Academy of Natural Science. He paid special attention to the collection and study of fungi and corresponded and exchanged with various mycologists, especially Curtis and Ravenel. He left a large collection of fungi, which the writers have recently had the privilege of examining. Among his specimens are found many labeled "Ex. Herb. Schw.", which are undoubtedly part of Schweinitz's original collections at the Philadelphia Academy. These specimens, as well as all of Michener's fungi, are mounted in exactly the same manner as the mounted portion of Schweinitz's collection at the Philadelphia Academy. The mounting paper, the specimen slips, the arrangement, manner of attachment, and the handwriting on the labels are identical, as will be readily perceived by comparing the illustrations from photographs of sheets from both herbaria. It is, therefore, clear that the mounted collection of Schweinitz's herbarium was prepared by Dr. Michener. He evidently took from Schweinitz's original paper packets what appeared to him to be the best or most typical specimen of the species in the packet and attached it with glue to a square slip of paper, as shown in Plate III. Where there was but little material in the original packet it was all mounted in this manner. In case there were several pieces in the original packet he used his own discretion in making the selection of the part to be mounted and the part to be left.

When there were included in the original packet specimens from different hosts or different localities, in some cases representing different species, it would have been difficult, if not impossible, to determine which was the original material from which Schweinitz's description was made. At the same time, Dr. Michener, in case the specimen was not too scanty, evidently took a small portion of it for his own herbarium. Michener's catalogue of his herbarium lists *Sphaeria gyrosa* Schw. Consulting his collection it is found that No. 1431, the number of Schweinitz's specimen, is missing. Pin holes in the mounting sheet, however, show that the specimen which was once there has been removed. As perhaps throwing some light on the possible location of this specimen, it may be said that a specimen apparently typical *S. gyrosa*, pycnidial form on beech, labeled by Dr. William Trelease as *Sphaeria gyrosa* from Pennsylvania, was seen in the Boissier Herbarium, Geneva. Dr. Trelease tells the writers that this specimen probably came from Dr. Michener, and as there is no evidence that Dr. Michener or any one else has collected *E. gyrosa* in Pennsylvania there is considerable probability that this specimen represents a portion of Schweinitz's original collection.

In most cases all of the material in Schweinitz's original species packets was removed and either mounted or distributed. This was the case with *Sphaeria gyrosa*. The original packet of Schweinitz, which was fortunately preserved with all the others, is empty and apparently a part at least of the specimen which it contained is found in the mounted collection as prepared by Michener. This consists of a single piece of bark shown in Plate VI, figure 1. From the evidence the writers have been able to gather from Schweinitz's manuscripts and correspondence, as well as from studies of his writings and specimens in other herbaria, it appears that this specimen is the one indicated on the original packet and also by Schweinitz (74, p. 206) as having been collected in New England and sent to him by Torrey. This, as shown by his correspondence, was after he had left North Carolina. The bark upon which the fungus grew is clearly not Fagus, Juglans, or Quercus, the hosts originally given for *S. gyrosa*, but apparently Acer. It is therefore not a part of the original specimens from Salem, N. C., upon which his description was based, and in reality is not *Sphaeria gyrosa*, but a species of Nectria, which Schweinitz incorrectly identified as *S. gyrosa*. Portions of this same specimen are found in Berkeley's herbarium at Kew and in the Curtis herbarium at Harvard. They are clearly the Nectria referred to above from Torrey. In this connection, it may be noted that E. Hitchcock in 1829 (42, p. 63) reports *Sphaeria gyrosa* Schw. from Amherst, Mass., and states in the preface to his list that Dr. Torrey assisted in the determination of the cryptogams.

"CANKERS" CAUSED BY ENDOTHIA PARASITICA ON CASTANEA DENTATA. × ½.

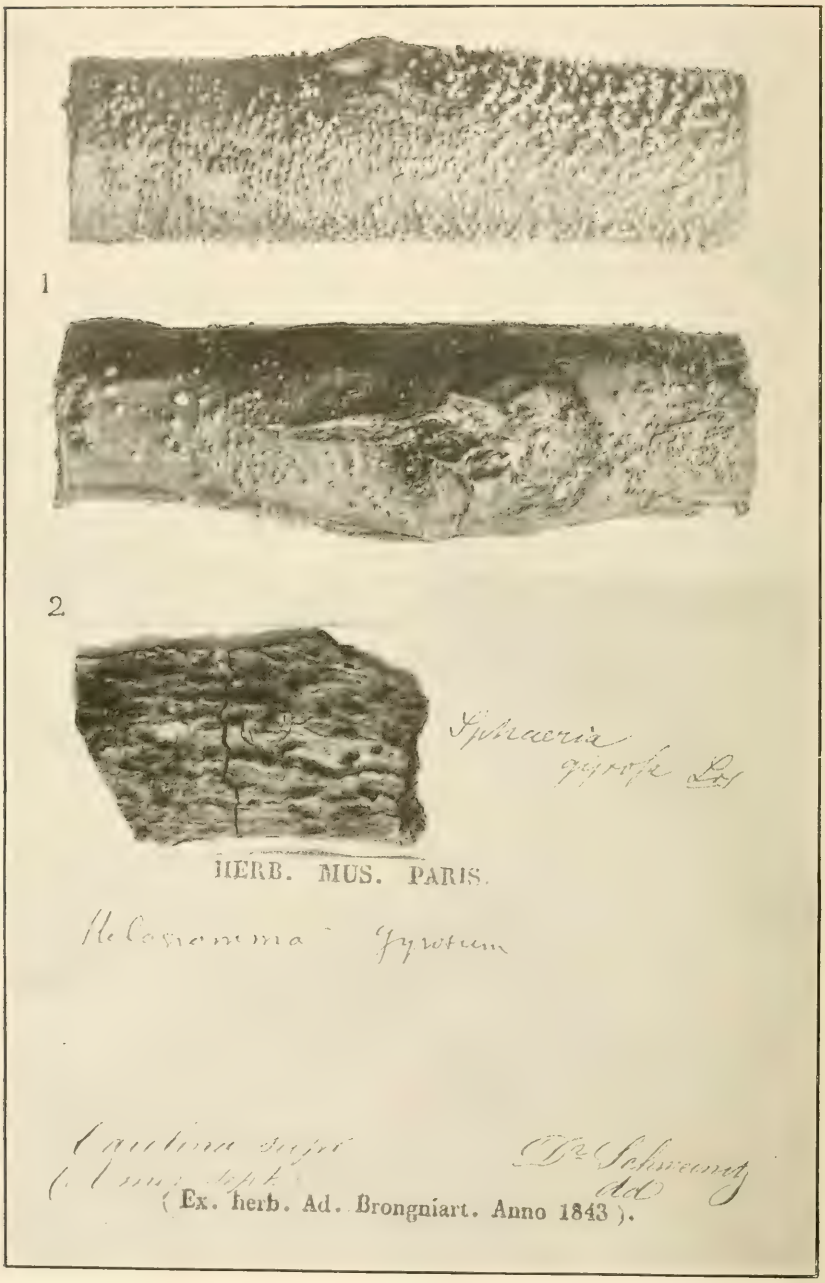
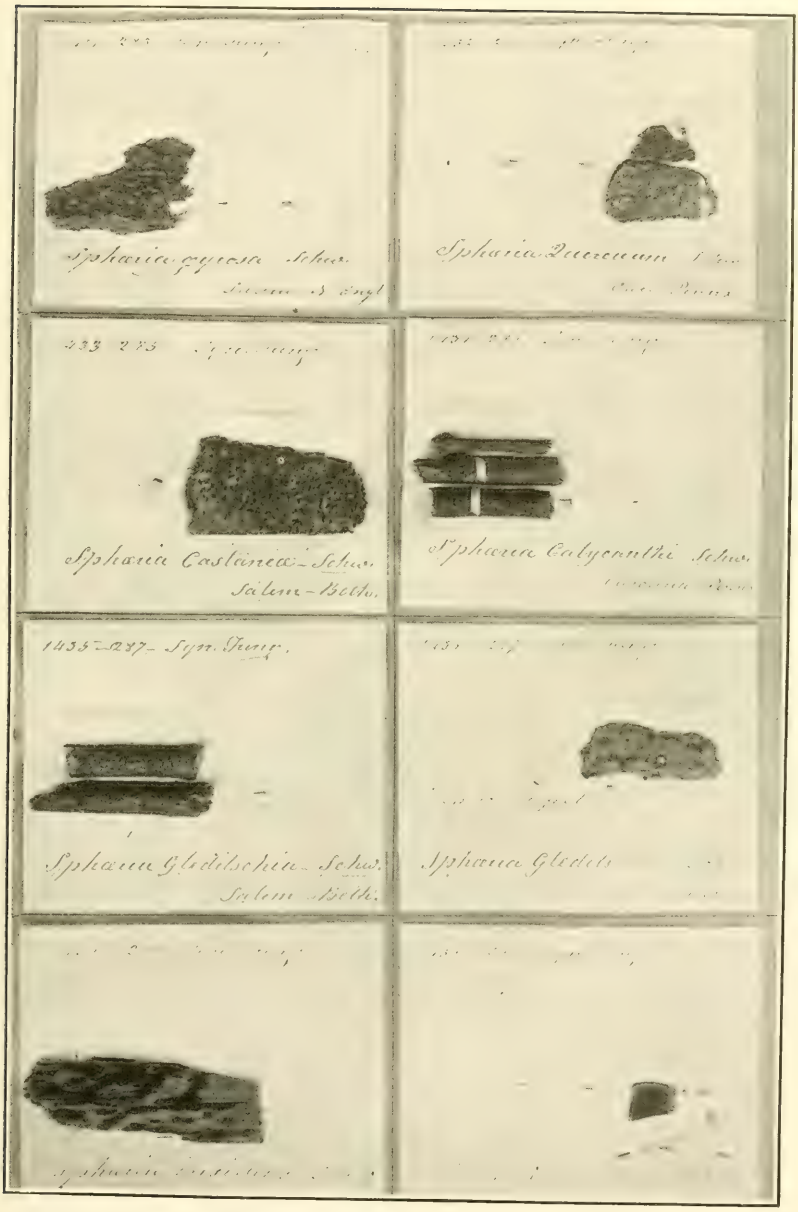
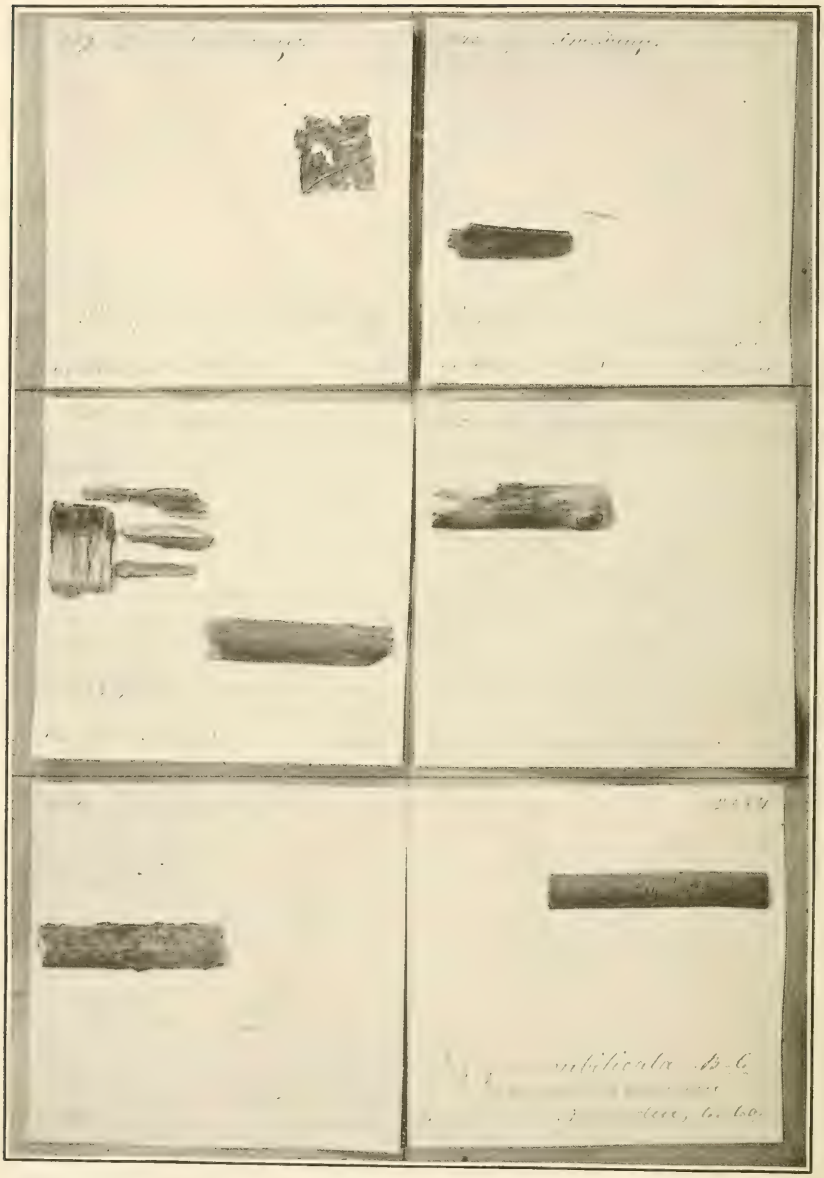



FIG. 1.—PERITHECIA AND PYCNIDIAL STROMATA OF ENDOTHIA PARASITICA WITH CANKERS ON CASTANEA DENTATA. FIG. 2.—COTYPE OF SPHAERIA GYROSA SCHW. ON FAGUS.

Specimen now in the Paris Museum sent to Brongniart by Schweinitz, showing Tulasne's label "*Melogramma gyrosum*" and Schweinitz's autograph label.




A SHEET FROM THE MOUNTED PORTION OF SCHWEINITZ'S HERBARIUM AT THE PHILADELPHIA ACADEMY OF SCIENCES, SHOWING SPECIMENS AS PREPARED AND LABELED BY MICHENER.



A SHEET FROM MICHENER'S HERBARIUM, SHOWING A PART OF SCHWEINITZ'S TYPE OF PEZIZA CINNABARINA (UPPER RIGHT HAND CORNER); ALSO SHOWING CLEARLY THAT THE MOUNTING AND LABELING OF THIS AND SCHWEINITZ'S COLLECTION WERE DONE BY THE SAME PERSON.

This seems to explain the origin of the specimen which Schweinitz received from Dr. Torrey. The writers have searched in vain for *Endothia gyrosa* in Amherst and vicinity and they know of no collections of the fungus from Massachusetts. No specimens upon which Hitchcock's list was based have been located.

Since it can be clearly shown that little or none of the original type collection of this species is in the Philadelphia Academy collection it must be looked for elsewhere. It is found by reference to Schweinitz's correspondence and manuscripts, which have been carefully examined by the writers through the courtesy of the Philadelphia Academy and the descendants of Schweinitz, and also by studies in foreign herbaria that he divided his specimens with many of his European and American correspondents. As he does not appear to have kept any duplicates separate from his regular collection it seems probable that the specimens he distributed were taken from the original packets. Thus in some cases, apparently all of a type specimen was removed from the original packet. In fact, in one instance (73, p. 5) he states that he sent his only specimen of a species of Hypoxylon to Dr. Schwaegrichen, of Leipzig.

It seems rather certain from statements made by Schwaegrichen in his introduction to Schweinitz's paper on the fungi of North Carolina (72) that specimens of a large number, if not all, of the species represented in that work were sent to him. The types or parts of the types should therefore be found in Schwaegrichen's herbarium. In spite of all their efforts, however, through correspondence and personal search in Europe, the writers have been unable to locate Schwaegrichen's collection of fungi. They found, however, in the herbarium of the University of Leipzig a small bit of a specimen labeled "*Sphaeria gyrosa* Schwein. Juglans Fagus Carolina D. Schwaegrich. dd 5–21 K. Z." This specimen is evidently a part of the original collection of Schweinitz which was sent to Schwaegrichen and given by him to Dr. Kunze. The host is apparently neither Juglans nor Fagus, but seems to be Quercus. It may be noted in this connection that in spite of diligent search by the writers and various other collectors no specimen of Endothia has yet been found on Juglans in this country. Neither have the writers been able to find any specimen in the various herbaria examined. They have concluded, as a result of their studies, that the mention of Juglans by Schweinitz was an error in the identification of the host, which it is believed was really Quercus, the host upon which *E. gyrosa* is most frequently found in the South, and especially in the vicinity of Salem. According to the American Code,[1] however, the specimen which should be taken as the type in this case is the one on

[1] American Code of Botanical Nomenclature. Canon 14, b. Bulletin, Torrey Botanical Club, vol. 34, p. 172. 1907.

Fagus, as this is the first-mentioned host in the original description. No specimen of this species on Fagus from Schweinitz was found in Kunze's collection. However, authentic specimens from Schweinitz on Fagus have been found in Fries's herbarium at Upsala, in Hooker's herbarium at Kew, and in Brongniart's herbarium in the Paris Museum. The last, which is the largest and best specimen, is shown in Plate II, figure 2. Microscopic studies of the specimens at Paris and Kew show only pycnidia with pycnospores. The writers were unable to examine microscopically the specimen in Fries's herbarium, but it agreed in all macroscopic respects and also, so far as could be determined with a hand lens, with the Paris and Kew specimens. These specimens agree with all the material collected on Fagus from various localities in the South. Studies of numerous collections of *E. gyrosa* have shown that the pycnidial form can be distinguished with certainty from any of the other species of Endothia at present known. The connection between this pycnidial form and the perithecial form as described has been demonstrated by pure cultures from ascospores and also by the association of typical pycnidia and pycnospores with perithecia and ascospores in the same stroma. There appears to be no reasonable doubt, therefore, that the specimens collected by Schweinitz on Fagus were the pycnidial form of *Endothia gyrosa*, and the specimen in the Paris Museum which was sent by Schweinitz to Brongniart about 1825 may properly be considered a cotype of Schweinitz's species. The specimen from Schweinitz in Kunze's herbarium at Leipzig also proves on microscopic examination to be the pycnidial form of the same fungus. It is probable from the evidence at hand that Schweinitz did not collect any specimen showing ascospores of this fungus. However, the specimen in Kunze's herbarium shows some perithecia evidently immature and without spores. A part of the specimen from Schweinitz in Fries's herbarium shows stromata on a piece of bark, evidently not Fagus, but probably Quercus. This also appears to be pycnidia only.

The specimen referred to by Clinton (18), which was found in the original packet of Schweinitz at Philadelphia with *Sphaeria enteromela*, is also undoubtedly the pycnidial form of *E. gyrosa*, which closely resembles some early stages in the development of species of Hypoxylon, especially *H. enteromela*. These species may be easily confused with each other, and this would seem to be a probable explanation of the accidental presence of this specimen in this packet. Another point of interest in this connection is the fact that in spite of diligent search on the part of the writers and many other collectors and an examination of numerous specimens of Endothia on Fagus in all stages of development and from different localities only *Endothia gyrosa* has been found on this host. Of

course, it can not be positively stated that *E. fluens* does not occur on Fagus in this country, but if it does it must be rare. In this connection, it is also perhaps worthy of note that, notwithstanding the mention of Fagus as a host in Europe, the writers have never seen any European specimens of Endothia on this host. The specimens so named by Roumeguere and distributed as No. 989 Fun. Gal. on beech are, according to several specimens examined, evidently a young condition of some Hypoxylon, probably *H. coccineum*, which in this state bears a superficial resemblance in form and color to the stromata of Endothia, but can be easily distinguished by the dark-brown or blackish color of the interior of the stroma. The identity of Schweinitz's *Sphaeria gyrosa* with the long ascospore form of Endothia shown on Plate VII is based on careful microscopic study of the stromata and measurement of the pycnospores from four specimens of the original collections of Schweinitz in North Carolina, three on Fagus and one labeled Juglans. The three on Fagus show the typical pycnidial stromata and pycnospores of the species, either of which is sufficient for positive identification when thoroughly known. The specimen referred to by Schweinitz as on Juglans also shows typical pycnospores of *E. gyrosa*. The evidence, as stated above, leaves no reasonable doubt as to the identity of the fungus which Schweinitz described as *Sphaeria gyrosa*.

According to a specimen which is probably a portion of Schweinitz's type found in Michener's herbarium, *Peziza cinnabarina* Schw. is the pycnidial form of *E. gyrosa* (Schw.) (See Pl. IV.) It is the form with small pycnidia on bare wood of Liquidambar. This was first reported by Schweinitz as "Peziza flammea A. and S." and later changed as above. Later Saccardo (69, vol. 8, p. 399), thinking that this was a Discomycete, transferred it to the genus Lachnella.

The other American species of Endothia which was described by Schweinitz as *Sphaeria radicalis* and first published by Fries in 1828 (31, p. 73) has also until recently been more or less misunderstood. The only specimens of this species found at present in Schweinitz's mounted collection at the Philadelphia Academy of Science is a small piece of bark of an oak root bearing a few pycnidial stromata. No host was given in Fries, but Schweinitz in 1832 (74 p. 197) gives Fagus as the host. That this was an error and that the host was really Quercus and not Fagus is clearly indicated by all of Schweinitz's specimens examined, not only those in the Philadelphia Academy but those found in several herbaria in Europe and one in Curtis's herbarium at Harvard, and also in Schweinitz's autograph label on the original packet in his herbarium. A photograph of this packet is shown in Plate VI.

The description of this species was first published by Fries in 1828 (31, p. 73). Schweinitz's specimen at the Philadelphia Academy

shows only pycnidia. (See Pl. V, fig. 2.) His description, however, as well as his unpublished illustrations preserved in the library of the Academy, show clearly that perithecia were present in the material from which the description was made. This is also conclusively shown by authentic specimens from Schweinitz in at least two European collections, those of Fries at Upsala and Hooker at Kew. A microscopic examination of these specimens shows good perithecia and mature ascospores having the characters and measurements given elsewhere in this paper for *Endothia fluens* (Sow.). (See Pl. XVII, fig. 9.) As there is no indication in Schweinitz's writings or in his manuscript notes and records that he made more than one collection of this species, there is no reason to doubt that the material at Upsala and Kew is a part of that upon which he based his description of *Sphaeria radicalis* Schw. The true type specimen of the species is that in Fries's herbarium upon which he based his description, which was added to the diagnosis sent by Schweinitz.

One year after the description of this species from America it was reported from Italy by Rudolph, in 1829 (66, p. 393), and in 1830 Fries (32, p. 541) himself reports the fungus from France. This species had, however, been collected and described before in its pycnidial condition in 1814 by Sowerby (79, pl. 438) under the name of *Sphaeria fluens*. This was reported in 1836 by Berkeley (8, p. 254) as *Sphaeria gyrosa* Schw. A microscopic study of the original material of this species, which was collected by Charles Lyell on chestnut in the New Forest in southern England and is now preserved in the Kew Herbarium, leaves no doubt that it is the pycnidial form of *Endothia radicalis* (Schw.). Plate XVII, figure 3, shows pycnospores from Sowerby's specimen at Kew. This specimen agrees with Sowerby's illustration and is apparently the one from which this figure was made. The pycnospore masses are somewhat larger than usual; otherwise it is typical of *E. radicalis* Schw.

At first it did not seem possible to distinguish the species of Endothia in their pycnidial condition, but thorough microscopic studies of large quantities of material in the field and laboratory in both America and Europe have shown that the two sections of the genus and some of the species can usually be separated with certainty in this stage of their development, as indicated by the tables of measurements and in the photographs of pycnospores, and especially by the stromata of the different species.

The first description of the ascospores of *E. radicalis* was given in 1858 by Currey (21, p. 272), who examined the specimens from Schweinitz in Hooker's herbarium at Kew. Currey figured what he believed to be four ascospores. Two are apparently typical *E.*

*fluens;* the other two are more than 1-septate and belong to some other organism. Cesati and De Notaris, in 1863 (11), first definitely referred *Sphaeria radicalis* Schw. to Endothia. Up to this time *Sphaeria gyrosa* and *Sphaeria radicalis* were generally regarded by mycologists as separate species and were placed by Schweinitz and Fries in different groups of the genus Sphaeria, though they both mention a similarity in external appearances.

In 1863 the Tulasnes, in their epoch-making work on the fungi (83, pp. 87–89), made a careful microscopic study of the specimens from Schweinitz preserved in the Paris Museum and also specimens received from De Notaris, Berkeley, and other collectors. At that time no ascospores of *Sphaeria gyrosa* had apparently been described by mycologists. The material of *S. gyrosa* from Schweinitz which the Tulasnes found in the Paris Museum included the specimen on Fagus which had been sent by Schweinitz to Brongniart. There seems to be no evidence that the Tulasnes examined other specimens from Schweinitz or that they examined any specimens showing ascospores of the true *Sphaeria gyrosa.* This is indicated by their description and measurements of the ascospores. From their studies of Schweinitz's specimens and from other Carolina specimens sent them by Berkeley they concluded that *Sphaeria gyrosa* and *Sphaeria radicalis* are the same species and called it *Melogramma gyrosum.*

Fries (33, pp. 385–386) had earlier (1849) reported *Sphaeria gyrosa* as occurring in southern Europe. This report was apparently based upon specimens of pycnidial stromata of *E. fluens*, somewhat larger and more irregular in shape than usual, collected in western France by Guepin and Levieux and already referred to.

The statement of the Tulasnes (83, pp. 84–89) in regard to the identity of these species was accepted by practically all mycologists down to 1912, when the discussion in regard to the origin and relationships of *Endothia parasitica* commenced. Ellis and Everhart in 1892 (26, p. 552) apparently figured the true *E. gyrosa* Schw. but cited exsiccati of both *E. gyrosa* and *E. fluens* and gave the ascospore characters and measurements of *E. fluens*, apparently copied from Winter (85, p. 803), as the spores figured do not agree with the description.

## THE SPECIES OF ENDOTHIA.

**ENDOTHIA Fries, 1849, Sum. Veg. Scand., p. 385.**[1]

SYNONYMS:

Endothiella Sacc., 1906, in Ann. Mycol., v. 4, no. 3, p. 273. Type species, *E. gyrosa* Sacc., 1 c.

Calopactis H. and P. Syd., 1912, in Ann. Mycol., v. 10, no. 1, p. 82. Type species, *C. singularis*, 1 c.

[1] All references to literature in synonymy are given in full in "Literature cited," p. 77.

Stromata subcorticular in origin, variable in size and shape, pustular to subspherical, subcoriaceous to friable, sometimes confluent, surface light auburn[1] or chestnut to mahogany red, capucine yellow or cadmium orange to scarlet within; pycnidial and perithecial stromata the same or similar; pycnidia few to numerous, consisting of simple cavities or complex and irregular chambers; pycnospores minute, simple, bacilliform to oblong, yellowish to reddish in mass; perithecia deeply immersed, in one or more irregular layers, usually black when mature, with long necks, black within, colored like the stroma without; asci clavate to oblong fusoid, 8-spored, usually without paraphyses; ascospores oblong fusoid or subellipsoid to cylindric or allantoid cylindric, uniseptate or nonseptate, hyaline to pale yellowish.

*Section 1.*—Ascospores short cylindric to allantoid, continuous or pseudoseptate.

**ENDOTHIA GYROSA (Schw.) Fries, 1849, Sum. Veg. Scand., p. 385. p. p.**

Synonyms:

Pycnidia: *Sphaeria gyrosa* Schw., 1822, Syn. Fung. Car. Sup., p. 29, no. 24.
*Peziza flammea* Schw. (not. Alb. and Schw.), 1822, Syn. Fung. Car. Sup., p. 93, no. 41, p. p. ad Liquidambar.
*Sphaeria gyrosa* Fries, 1822, Syst. Mycol., v. 2, p. 419.
*Peziza gyrosa* Spreng., 1827, Syst. Veg., v. 4, pars. 1, p. 515.
*Peziza cinnabarina* Schw., 1832, Syn. Fung. Am. Bor., p. 173.
*Melogramma gyrosum*, L. R. and C. Tul., 1863, Selecta Fung. Carpol., t. 2, p. 87. p. p. min.
*Melogramma gyrosum* M. A. Curtis, 1867, Cat. Indig. Nat. Plants, p. 143.
*Endothia gyrosum* (Tul.) Fckl., 1869, Symb. Mycol., p. 226.
*Melogramma gyrosum* Tul., Rav., 1879, Fung. Amer. Exs., no. 352.
*Lachnella cinnabarina* Sacc., 1889, Syll. Fung., v. 8, p. 399.

Perithecia: *Sphaeria gyrosa* Schw., Rav., 1852, Fung. Car. Exs., no. 49.
*Melogramma gyrosum* Tul., Cooke, 1878, *in* Ann. N. Y. Acad. Sci., v. 1, no. 5/6, p. 185.
*Endothia gyrosa* Fckl., Sacc., 1882, Syll. Fung., v. 1, p. 601. p. p. min.
*Endothia gyrosa* Schw., Ell. and Ev., 1887, No. Amer. Fung. Exs., no. 1956.
*Endothia gyrosa* (Schw.) Ell. and Ev., 1892, No. Amer. Pyren., p. 552, p. p.
*Endothia radicalis* (Schw.) Farl., Clint., 1912, *in* Science, n. s., v. 36, no. 939, p. 908.
*Endothia radicalis* (Schw.) Shear, 1912, *in* Phytopathology, v. 2, no. 5, p. 211.
*Endothia radicalis* (Schw.) Fries, P. J. and H. W. And., 1912, *in* Phytopathology, v. 2, no. 5, p. 210.

Type specimen.—The type in Herb. Schw. is wanting. A cotype is in Herb. Museum of Paris.

Pycnidia.—Stromata corticular or subcorticular, pulvinate to tubercular, rugulose, scattered or gregarious, occasionally confluent, 1.5 to 3 mm. in diameter by 1.5 to 2 mm. high, orange chrome when young to chestnut when mature,

[1] In the following descriptions of cultures and elsewhere throughout this paper, the names of colors are taken from Ridgway's recent work on color nomenclature (64).

becoming almost black when old and weathered, cadmium orange within; pycnidia consisting of numerous irregular labyrinthiform chambers in the stroma, separated by walls of varying thickness and opening by irregular pores in the surface of the stroma; sporophores cylindric or slightly tapering toward the apex, 6 to 9 $\mu$ long; pycnospores oblong, straight or sometimes slightly curved, appearing hyaline when separate, warm buff to ochraceous buff or darker, according to mass and moisture content, 3 to 4 by 1.5 to 2 $\mu$.

Perithecia.—Stromata the same or similar to those producing pycnidia; perithecia dark, membranous, few to many, mostly 25 to 50, usually arising in the lower portion of the stroma, 150 to 300 $\mu$ in diameter, very irregularly arranged in one to several layers, prolonged into slender necks which penetrate the stroma above and sometimes protrude somewhat, terminating in a short conical ostiole; asci oblong fusoid or subclavate, very short stipitate, 25 to 30 by 6 to 7 $\mu$; ascospores irregularly biseriate, cylindric to allantoid, 7 to 11 by 2 to 3 $\mu$, mostly 7.5 to 10 by 2 to 2.5 $\mu$, hyaline when separate, slightly yellowish in mass, with a very thin gelantinous envelope when mature.

Cultural characters.—Cultures one month old on white corn meal show an abundant thick growth of mycelium producing irregular tubercular masses resembling pycnidial stromata, but without spores. The surface color is capucine buff. The medium usually changes to perilla purple. It is distinguished from *E. singularis*, its nearest relative, by its more rapid growth and the formation of the large tubercular masses.

Hosts.—Exposed roots and branches: *Quercus alba, Q. coccinea, Q. falcata, Q. georgiana, Q. ilicifolia, Q. imbricaria, Q. marylandica, Q. nigra, Q. phellos, Q. prinus, Q. rubra, Q. velutina, Q. virginiana, Liquidambar styraciflua, Fagus americana* and *F. sylvatica* cult. vars., *Castanea dentata* and cult. vars., and *Vitis* sp. (25).

A specimen of this species collected by Ravenel has the host given as maple (Acer), but microscopic examination shows it to be Liquidambar.

Type locality.—Salem, N. C.

Geographical distribution.—Southwestern Connecticut to central Michigan, southward to Florida and Texas; also Kansas and California.

Illustrations.—Ell. and Ev., 1892, No. Amer. Pyren., pl. 36, fig. 68; Clint. 1913, *in* Conn. Agr. Exp. Sta. Rpt., 1911, 1912, pl. 28, fig. a, d, and g.

Exsiccati.—Pycnidia: Baker, Pl. Pac. slope, 722, on *Quercus agrifolia;* Rav. Fung. Amer., 352, on Quercus. Perithecia: Ell. and Ev. No. Amer. Fung., 1956, on Quercus; Rav. Fung. Car., 49, on Quercus and Liquidambar.

### ENDOTHIA SINGULARIS (H. and P. Syd.) S. and S. nov. comb.

Synonyms:

Pycnidia: *Calopactis singularis* H. and P. Syd., 1912, *in* Ann. Mycol., vol. 10, no. 1, p. 82.

*Endothia gyrosa* Ell. and Ev., in Herb. N. Y. Bot. Gard.

*Endothia gyrosa* (Schw.) Fckl. Höhnel, 1913, *in* Sitzber, K. Akad. Wiss. [Vienna], Math. Naturw. Kl., Abt. 1, Bd. 122, Heft 2, p. 298.

Type specimen.—H. and P. Syd., Fung. Exot, no. 88, on *Q. gambellii.*

Pycnidia.—Stromata corticular, erumpent, depressed globose, sometimes irregular, scattered, or gregarious, 3 to 5 mm. wide by 2 to 4 mm. high, outer wall thick, coriaceous, becoming brittle, mahogany red without, scarlet within; pycnidia consisting of innumerable nearly spherical cavities throughout the stroma, 25 to 35 $\mu$ in diameter, the walls disintegrating into a powdery mass and the whole set free by the irregular rupture of the stroma wall, usually leaving a cup-like basal portion attached to the bark; sporophores, according to the Sydows,

short, hyaline, subulate, 6 to 8 by 1 $\mu$; pycnospores ovoid oblong, hyaline, the contents of each pycnidial cavity adhering in a globular mass, when set free, 3 to 4 by 1 to 1.5 $\mu$.

PERITHECIA.—Stromata the same or similar to those producing pycnidia; perithecia membranous, few to many, usually 100 or more, 200 to 350 $\mu$ in diameter, irregularly arranged in several series, prolonged into slender necks which sometimes protrude from the stroma; ostioles depressed conical; asci, oblong cylindric or subclavate to fusoid, substipitate, 25 to 35 by 4.5 to 5.5 $\mu$; ascospores irregularly biseriate, cylindric to allantoid, with a thin gelatinous envelope, hyaline when separate, slightly yellowish in mass, 7 to 11 by 1.5 to 3 $\mu$; mostly 7.5 to 10 by 2 to 2.5 $\mu$.

CULTURAL CHARACTERS.—Cultures one month old on white corn meal have a cadmium and orange to capucine buff mycelium. It is distinguished from *E. gyrosa* by its slower growth and brighter color and the want of tubercular, stromalike masses. No spores of this species have been produced in any of the writers' cultures.

HOSTS.—*Quercus gambellii, Q. leptophylla, Q. nitescens, Q. utahensis.* Bethel also reports it on *Q. pungens.*

TYPE LOCALITY.—Palmer Lake, Colo.

GEOGRAPHICAL DISTRIBUTION.—Colorado and New Mexico.

ILLUSTRATIONS.—Pycnidia: H. and P. Syd., 1912, *in* Ann. Mycol., vol. 10, no. 1, p. 82, figs. 1–5.

EXSICCATI.—Pycnidia and perithecia: H. and P. Syd., Fung. Exot., 88, on Quercus. Pycnidia: Bart. Fung. Col., 4002, on *Quercus utahensis.*

In shape and size of pycnospores and ascospores this species closely resembles *E. gyrosa*, but is easily separated by the much greater size of its stromata, its brighter color and very numerous, small, regular pycnidial cavities and more numerous perithecia, as well as its geographical distribution.

The specimens of the Sydow exsiccati, No. 88, in the Pathological and Mycological Collections of the Bureau of Plant Industry show both pycnidia and perithecia.

*Section 2.*—Ascospores oblong fusiform to oblong ellipsoid, uniseptate when mature.

**ENDOTHIA FLUENS (Sow.) S. and S. nov. comb.**

SYNONYMS:

Pycnidia: *Sphaeria fluens* Sow., 1814, Col. Fig. Engl. Fungi, Sup. pl. 438, figs. 1, 2.

*Sphaeria gyrosa* Berk., 1836, Brit. Fungi, p. 254. Not Schw.

*Endothia gyrosa* Fries, 1849, Sum. Veg. Scand., p. 385. p. p. Europ.

*Sphaeria radicalis* Fckl., 1861, Enum. Fung. Nass., p. 76, no. 640.

*Endothia gyrosum* Fckl., 1869, Symb. Mycol., p. 226. p. p. spec. cit.

*Endothia gyrosa* (Schw.) Fckl., forma *castaneae vescae* Sacc., 1876, Mycol. Ven. Exs., no. 929.

*Endothiella gyrosa* Sacc., 1906, *in* Ann. Mycol., v. 4, no. 3, p. 273.

Perithecia: *Sphaeria radicalis* Schw., Fries, 1828, Elenchus Fung., v. 2, p. 73.

*Sphaeria radicalis* Schw., Rudolphi, 1829, *in* Linnaea, Bd. 4, Heft 3, p. 393.

*Sphaeria radicalis* Schw., Fries, 1830, *in* Linnaea, Bd. 5, Heft 4, p. 541.

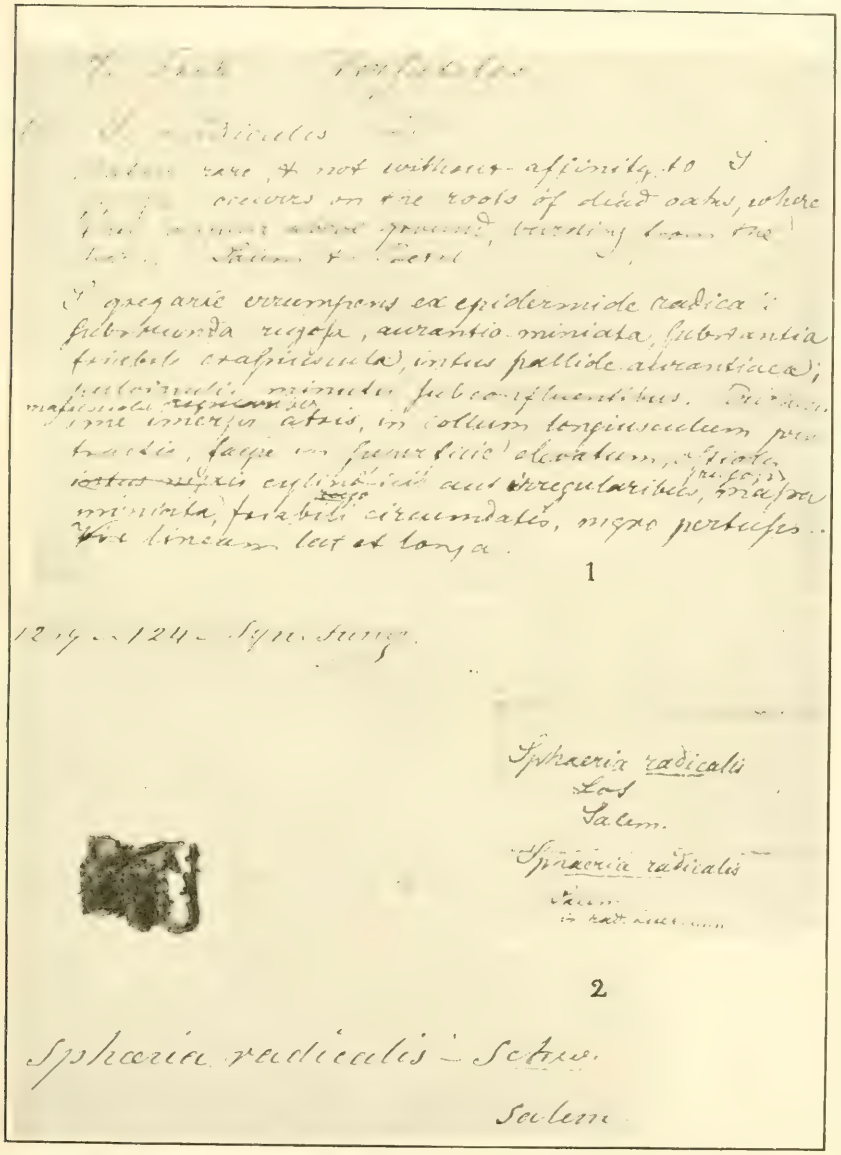



FIG. 1.—PHOTOGRAPH OF SCHWEINITZ'S MANUSCRIPT NOTES, WITH HIS DESCRIPTION OF SPHAERIA RADICALIS.' FIG. 2.—SPECIMEN OF S. RADICALIS IN THE MOUNTED COLLECTION OF SCHWEINITZ, AS PREPARED BY MICHENER; ALSO ORIGINAL PACKET WITH SCHWEINITZ'S AUTOGRAPH LABEL.

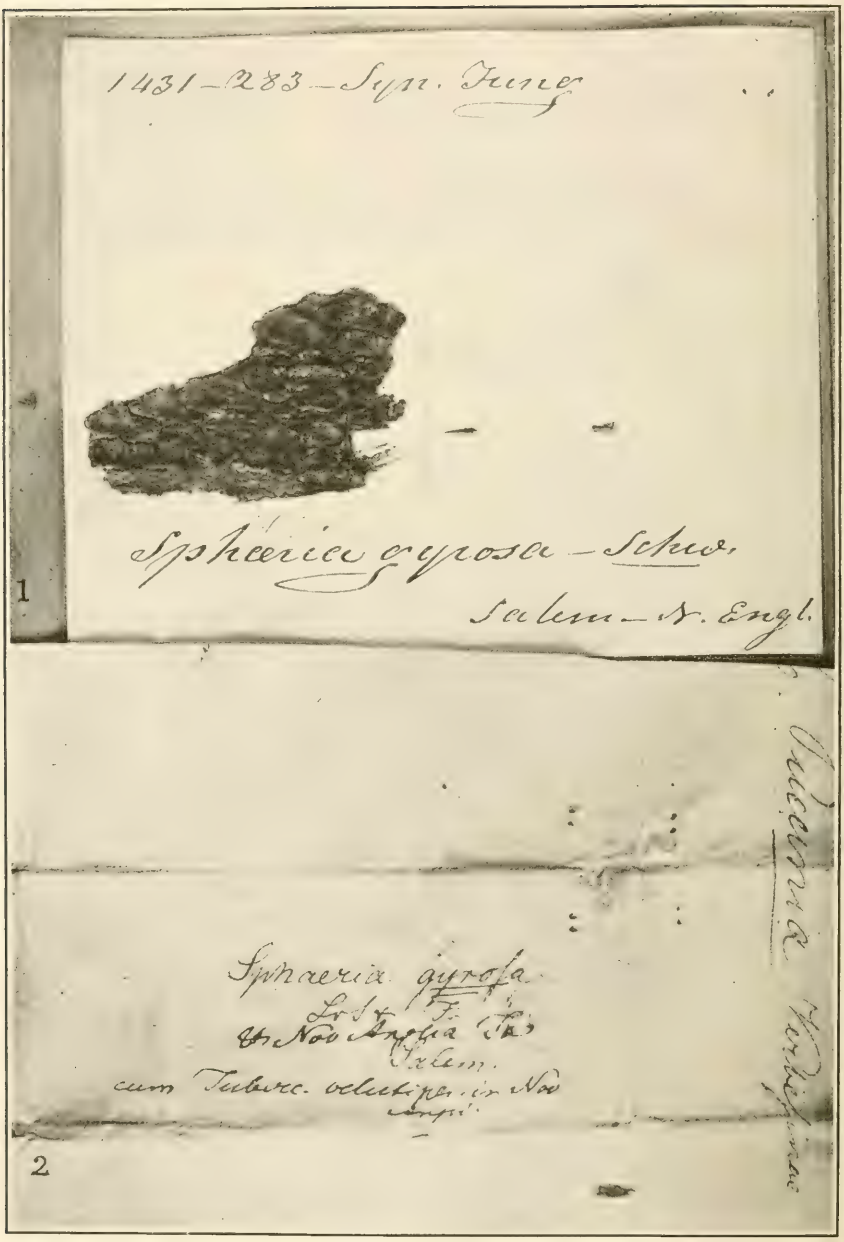



FIG. 1.—PHOTOGRAPH OF THE SPECIMEN IN SCHWEINITZ'S HERBARIUM MOUNTED BY MICHENER. NOT TRUE ENDOTHIA GYROSA BUT A NECTRIA. FIG. 2.—ORIGINAL PAPER PACKET IN WHICH SCHWEINITZ'S TYPE MATERIAL OF E. GYROSA WAS PRESERVED, WITH HIS AUTOGRAPH LABEL.

ENDOTHIA GYROSA GROWING ON THE RECENTLY CUT END OF A LIVING BRANCH OF FAGUS SP. NATURAL SIZE.

MYCELIAL FANS OF ENDOTHIA PARASITICA UNDER THE BARK OF CASTANEA DENTATA.

Illustration from Heald (39), by courtesy of I. C. Williams, Pennsylvania State Forestry Department.

Synonyms—Continued.

Perithecia—Continued.

*Sphaeria radicalis* Schw., 1832, Fun. Am. Bor., p. 197.

*Sphaeria radicalis* Schw., Mont., 1834, *in* Ann. Sci. Nat. Bot., s. 2, t. 1, p. 295.

*Sphaeria* (Diatrype) *radicalis* Fries, Currey, 1858, *in* Trans. Linn. Soc. London, v. 22, pt. 3, p. 272, pl. 47, fig. 89. p. p.

*Valsa radicalis* Ces. and De Not., 1863, *in* Comm. Soc. Crittog. Ital., v. 1, p. 207.

*Endothia radicalis* (Schw.) Ces. and De Not., 1863, *in* Comm. Soc. Crittog. Ital., v. 1, opp. p. 240.

*Melogramma gyrosum* L. R. and C. Tul., 1863, Selecta Fung. Carpol., t. 2, p. 87. p. p. max.

*Sphaeria* (Diatrype) *radicalis* Schw., Currey, 1865, *in* Trans. Linn. Soc. London, v. 25, pt. 2, p. 244.

*Endothia gyrosa* (Schw.) Fckl., Sacc., 1882, Syll. Fung., v. 1, p. 601. p. p.

*Endothia gyrosa* var. *rostellata* Sacc., 1882, Syll. Fung., v. 1, p. 602.

*Endothia radicalis* (Schw.) Wint., 1887, Pilze, p. 803.

*Endothia gyrosa* Schw., Ell. and Ev., 1892, No. Amer. Pyren., p. 552. p. p.

*Endothia virginiana* P. J. and H. W. And., 1912, *in* Phytopathology, v. 2, no. 6, p. 261.

*Endothia gyrosa* (Schw.) Fries, Clint., 1913, *in* Conn. Agr. Exp. Sta. Rpt., 1911–12, p. 425.

*Endothia pseudoradicalis* Petri, 1913, *in* Atti R. Accad. Lincei Rend. Cl. Sci. Fis., Mat. e Nat., s. 5, v. 22, sem. 1, fasc. 9, p. 654.

*Endothia gyrosa* (Schw.) Fckl., Höhnel, 1913, *in* Sitzber. K. Akad. Wiss. [Vienna], Math. Naturw. Kl., Abt. 1, Bd. 122, Heft 2, p. 298.

Type specimen.—Sowerby in Herb. Kew. on *Castanea sativa*, New Forest, England. Coll. C. Lyell, Apr. 15, 1809.

Pycnidia.—Stromata corticular or subcorticular, truncate conical to pulvinate, usually separate and gregarious, but frequently confluent, 0.75 to 3 mm. in diameter by 0.5 to 2.5 mm. high, compact, varying from light auburn to chestnut on the surface and capucine yellow to cadmium orange within; pycnidia consisting of simple or more or less complex and irregular chambers in the stroma, opening by an irregular pore or slit at the apex of the stroma; sporophores usually simple, sometimes branched near the base, cylindric to subclavate, 10 to 13 $\mu$ long, sometimes 24 to 30; pycnospores oblong to rod-like, pale yellowish in mass, 3 to 5 by 1.5 to 2 $\mu$, mostly 3.5 to 4 by 2 $\mu$.

Perithecia.—Stromata the same or similar to those producing pycnidia; perithecia membranous, few to many, mostly 15 to 25, 300 to 400 $\mu$ in diameter, usually arising in the lower portion of the stroma, irregularly arranged in one to three layers, prolonged into slender necks which penetrate the stroma above and protrude usually from 300 to 600 $\mu$, terminating in conical ostioles; asci oblong fusoid or subclavate, very short stipitate, 30 to 40 by 6 to 8 $\mu$, mostly 30 to 35 by 7 $\mu$, ascospores irregularly biseriate, oblong fusoid or subellipsoid, not constricted at the septum, hyaline with a thin gelatinous envelope, 6 to 10 by 3 to 4.5 $\mu$, mostly 6.5 to 9 by 3 to 4 $\mu$.

Cultural characters.—Cultures one month old on white corn meal show a compact growth with a nearly smooth surface. The color ranges from light cadmium to empire yellow, and the medium becomes perilla purple. Pycnidia and spores usually appear a little later, forming large erumpent stromata which extrude thick masses of pycnospores. The light mycelium with large

pycnidial stromata and spore masses are distinguishing characters on this medium.

HOSTS.—America: Exposed roots and branches of *Q. alba, Q. coccinea, Q. marylandica, Q. prinus, Q. rubra, Q. velutina,* and *Castanea dentata.* Europe: Specimens examined, *Quercus pedunculata, Castanea sativa, Alnus glutinosa, Ulmus campestris, Carpinus betula,* and *Corylus* sp. Japan: *Castanea* sp. and *Pasania* sp. It is also reported on *Aesculus, Fagus,* and *Juglans* by Traverso.

TYPE LOCALITY.—New Forest, England.

GEOGRAPHICAL DISTRIBUTION.—America: Southern Pennsylvania and Ohio to South Carolina and northern Mississippi. Europe: Southern England, France, South Germany, and Switzerland to southern Italy and Transcaucasia. Asia: Japan.

ILLUSTRATIONS.—Sowerby, 1814, Col. Fig. Engl. Fungi, Sup., pl. 438; Currey, 1858, *in* Trans. Linn. Soc. London, v. 22, pt. 3, pl. 47, fig. 89 (2 upper spores); Ces. and De Not., 1863, *in* Comm. Soc. Crittog. Ital., pl. 3; Sacc., 1873, *in* Atti Soc. Veneto-Trentina Sci. Nat. Padova, v. 2, fasc. 1, pl. 14, fig. 63–65; Sacc., 1883, Gen. Pyren., pl. 6, fig. 6; Ruhl., 1900, *in* Hedwigia, Bd. 39, pl. 2, fig. 10; Trav., 1906, *in* Soc. Bot. Ital. Fl. Ital. Cript., pars 1, v. 2, fasc. 1, p. 180, fig. 34; P. J. and H. W. And., 1913, *in* Penn. Chestnut Tree Blight Com. Bul. 4, p. 22, fig. 2, A and C; Clint., 1913, *in* Conn. Agr. Exp. Sta. Rpt., 1911–12, pl. 28, fig. b, e, h, and j; Petri, 1913, *in* Atti R. Accad. Lincei Rend. Cl. Sci. Fis., Mat. e Nat., v. 22, sem. 1, fasc. 9, p. 656, fig. 1–3.

EXSICCATI.—Pycnidia: Thüm. Myc. Univ., 769, on Castanea; Sacc. Myc. Ven., 670, on *Carpinus betula;* Sacc. Myc. Ven., 929, on Castanea. Perithecia: Fckl. Fun. Nass., 640, on *Ulmus campestris;* Erb. Critt. Ital., 986, on Castanea; Rab. Herb. Viv. Myc., 254, on Castanea.

Roum. Fun. Sel. Gal., 989, labeled *Endothia gyrosa* Schw. on beech is apparently young *Hypoxylon coccineum.*

The most important synonyms given here have already been discussed. Of the others the writers have examined the types or collections upon which the identifications were based. All the material of Endothia in the herbaria of Cesati, De Notaris, Fuckel, and Berkeley, as well as other smaller collections, has been carefully studied. *E. virginiana* And. and And. has been studied in cultures, as well as typical specimens from the authors of the species, and agrees in every particular with *E. fluens.*

Through the kindness of Dr. Petri a part of the type of his *E. pseudoradicalis* has been examined, but unfortunately no cultures could be obtained from the specimen. The writers have been unable to distinguish his specimen from forms of *E. fluens* which appear to show all the intermediate conditions of variation connecting it with typical *E. fluens.* The ascospores of *E. fluens* are more variable in size and shape than those of any other species of Endothia studied. After examining many specimens of this species from Europe, it does not seem possible at present to separate any of them. The case of *E. pseudoradicalis* can not perhaps be regarded as closed until more material of it has been collected and compared in culture. In fact, the slide from the type of *Sphaeria radicalis* Schw. shows ascospores of both the narrow and broad form. The photomicro-

graph, Plate XVII, fig. 9, shows an ascospore which agrees with Petri's description and figures.

### ENDOTHIA FLUENS MISSISSIPPIENSIS S. and S. nov. comb.

SYNONYM:

*Endothia radicalis mississippiensis* Shear and Stevens *in* U. S. Dept. Agr., Bur. Plant Indus. Cir. 131, p. 4. 1913.

TYPE SPECIMEN.—No. 1782, on *Castanea dentata*, Blue Mountain, Miss., N. E. Stevens, Feb. 13, 1913. Deposited in Pathological and Mycological Collections, Bureau of Plant Industry.

CULTURAL CHARACTERS.—Cultures one month old on white corn meal show a compact, rather uniform surface, the color of the mycelium varying from cadmium orange to xanthine orange. This variety is distinguished from the species by the color of its mycelium, by the numerous small pycnidia thickly scattered over the surface of the culture, and by the lack of any purple color in the medium.

HOSTS.—*Castanea dentata*, *Quercus alba*, and *Q. velutina*.

GEOGRAPHICAL DISTRIBUTION.—Northern Mississippi, Kentucky, Tennessee.

COLLECTIONS EXAMINED.—On *Castanea dentata:* No. 1706 A. pycnidia, Corinth, Miss., T. E. Snyder; no. 708, pycnidia, Dumas, Miss., T. E. S.; no. 1782, ascospores, Blue Mountain, Miss., N. E. S.; no. 1806, ascospores, Blue Mountain, Miss., N. E. S. On Quercus: No. 1989, pycnidia, Danville, Ky., N. E. S.; no. 1995, pycnidia, Danville, Ky., N. E. S.; no. 2032, pycnidia, Lexington, Tenn., N. E. S.; no. 2255, pycnidia, Sardis, Miss., S. and S.

No morphological characters have yet been found to distinguish this variety. It is therefore separated on its cultural characters, which are marked and constant. The plant was first collected by T. E. Snyder, of the Bureau of Entomology.

### ENDOTHIA LONGIROSTRIS Earle, 1900, in Muhlenbergia, v. 1, no. 1, p. 14.

SYNONYM:

Perithecia: *Diatrype radicalis* (Schw.) Fries, Mont., 1855, *in* Ann. Sci. Nat. Bot. 4, t. 3, p. 123. Not Schw.

TYPE SPECIMEN.—No. 4340. A. A. Heller, Plants of Porto Rico. In Herb. N. Y. Bot. Garden.

PYCNIDIA.—Stromata corticular, erumpent, gregarious, sometimes confluent, 1 to 3 mm. in diameter, subcoriaceous, surface orange rufous to chestnut, interior zinc orange; pycnidia consisting of irregular labyrinthiform cavities opening by a single large pore or irregular rupture at the apex of the stroma; sporophores slender, somewhat tapering upward, mostly 8 to 10 $\mu$ long; pycnospores oblong elliptic, hyaline or yellowish in mass, when expelled forming a stout spore horn or tendril, colored like the stroma on the outside, 2 to 4 by 1 to 1.5 $\mu$.

PERITHECIA.—Stromata the same as those producing pycnidia, but larger and frequently confluent, forming linear series in crevices in the bark; perithecia arising usually at the base of the pycnidial stroma, mostly 3 to 10 in the separate stromata, membranous, 300 to 400 $\mu$ in diameter, mostly in a single irregular series, prolonged into long necks, 1.5 to nearly 1 cm. long, sec. Earle, internally black, externally same color and structure as the stroma; ostiole acute; asci oblong cylindric to fusiform, 25 to 35 by 5 to 7 $\mu$, mostly 30 by 6 $\mu$; ascospores overlapping uniseriate to irregularly biseriate, hyaline, ovoid to ovoid elliptical, 6 to 8.5 by 3 to 4 $\mu$, mostly 7 to 7.5 by 3 to 3.5 $\mu$.

CULTURAL CHARACTERS.—Cultures one month old on white corn meal have a uniform cadmium orange to xanthine orange color. The entire surface is covered with a compact growth, irregularly ridged. Tiny mars orange spore masses are scattered irregularly over the surface. Cultures of this species closely resemble *E. fluens mississippiensis* on this medium, being distinguished by the smaller and much less numerous spore masses. The medium is changed to amber brown just below the mycelium, shading into mars yellow; whereas, in the case of *E. fluens mississippiensis* the color of the medium is very little changed.

TYPE LOCALITY.—"Calcareous hills east of Santurce, Porto Rico, altitude 10 ft."

GEOGRAPHICAL DISTRIBUTION.—Porto Rico and French Guiana.

EXSICCATI.—Pycnidia and perithecia: Heller, Plants of Porto Rico, no. 4340.

This species, which appears to be subtropical or tropical in its range, is known at present from only three collections, the type collection from Porto Rico, a collection by Prof. N. Wille, No. 816, Porto Rico, distributed by the New York Botanical Garden, from which the cultures were obtained; and one made by Leprieur, No. 392, in French Guiana, and determined by Montagne as *Diatrype radicalis* (Schw.). A specimen of this collection apparently labeled by Montagne and preserved in the Delessert Herbarium at Geneva has been examined and found to agree with the type material of *E. longirostris*. It is readily distinguished from *E. tropicalis* by its smaller ascospores and pycnospores, and from *E. fluens* by its narrower and more acute ascospores and the long, slender necks of the perithecia.

**ENDOTHIA TROPICALIS Shear and Stevens sp. nov.**

SYNONYMS:

*Diatrype gyrosa* Berk. and Broome, 1875, *in* Jour. Linn. Soc. [London], v. 14, p. 124.

*Nectria gyrosa* Berk. and Broome, 1877, *in* Jour. Linn. Soc. [London], v. 15, p. 86.

*Cryphonectria gyrosa* (Berk. and Broome) Sacc., *in* Syll. Fung., v. 17, p. 784. 1905.

*Endothia gyrosa* (Schw.) Fckl., Höhnel, 1909, *in* Sitzber. K. Akad. Wiss. [Vienna], Math. Naturw. Kl., Abt. 1, Bd. 118, Heft 9, p. 1480.

TYPE SPECIMEN.—No. 2807 S. and S., on *Elacocarpus glandulifer*, Hakgala, Ceylon, Coll. T. Petch, August, 1913.

PYCNIDIA.—Stromata corticular, pustular to pulvinate, usually gregarious or scattered, rarely confluent, 1 to 5 mm. in diameter, early becoming friable, orange chrome when fresh to sanford brown when old and weathered; pycnidia consisting of numerous irregular cavities in the stroma; sporophores mostly simple, clavate, tapering above, 6 to 10 $\mu$ long.; pycnospores continuous, oblong to cylindric, very variable in size and shape, pale yellowish in mass, 3.5 to 7 by 1.5 to 2.5 $\mu$.

PERITHECIA.—Stromata the same or similar to those bearing pycnidia; perithecia black, membranous, collapsing when dry, 5 to 50 or more in a stroma; 250 to 500 $\mu$ diameter, irregularly arranged in one to three layers, bearing slender necks which penetrate the stroma and project 0.25 to 1 mm., terminating in acute ostioles; asci oblong or subclavate, nearly sessile, 40 to 50 by 7 $\mu$; ascospores irregularly biseriate, subelliptical, obtuse, not constricted at

the septum, hyaline with a gelatinous envelope, 7.5 to 10.5 by 3.5 to 5 μ, mostly 8 to 10 by 4 to 4.5 μ.

CULTURAL CHARACTERS.—Cultures one month old on white corn meal show small numerous, thickly scattered pycnidia and spore masses very similar to *E. parasitica*. The mycelium is orange buff to apricot orange. This species differs from *E. parasitica* in culture, chiefly in the brighter color of its mycelium.

HOST.—Rotten logs and stumps of *Elaeocarpus glandulifer*.

TYPE LOCALITY.—Hakgala, Ceylon.

GEOGRAPHICAL DISTRIBUTION.—Only known from Ceylon at present. One other collection of this species, No. 290 G. H. K. T. [Thwaite], N. Eliya, Ceylon, 6,000 feet, has been examined in the Kew Herbarium.

Through the kindness of Mr. T. Petch, of Peredeniya, the writers have received two large collections of this fungus. Some of the material was in a living condition and enabled the writers to obtain pure cultures for comparison with the other species of Endothia. This species is closely related to *E. parasitica*, but is readily separated by its larger ascospores and larger and more variable pycnospores and its nonparasitic habit.

**ENDOTHIA PARASITICA (Murr.) P. J. and H. W. And., 1912, in Phytopathology, v. 2, no. 6, p. 262**

SYNONYMS:

*Diaporthe parasitica* Murrill, 1906, *in* Torreya, v. 6, no. 9, p. 189.

*Valsonectria parasitica* Rehm, 1907, Asc. Exs., no. 1710.

*Valsonectria parasitica* Rehm, 1907, *in* Ann. Mycol., v. 5, no. 3, p. 210.

*Endothia gyrosa* var. *parasitica* Clint. 1912, *in* Science, n. s., v. 36, no. 939, p. 913.

*Endothia gyrosa* (Schw.) Fckl. Höhnel, 1909, *in* Sitzber. K. Akad. Wiss. [Vienna], Math. Naturw. Kl., Abt. 1, Bd. 118, Heft 9, p. 1480.

TYPE SPECIMEN.—Herbarium N. Y. Bot. Garden, on *Castanea dentata*, Bronx Park, New York City, Nov. 26, 1905, Coll. W. A. Murrill.

PYCNIDIA.—Stromata corticular, slightly erumpent to truncate conical, usually separate and gregarious, frequently confluent in more or less linear series especially in old rimose bark, 0.75 to 3 mm. in diameter by 0.5 to 2.5 mm. high, varying from capucine yellow when young to auburn when old and weathered; pycnidia consisting of irregular cavities in the stroma, 100 to 300 μ in diameter; sporophores mostly simple, subclavate, acute at the apex, usually 12 to 20 by 1.5 μ, more elongated filaments sometimes reaching 50 μ or more being frequently found among the normal sporophores; pycnospores, oblong to cylindric, rounded at the ends, 3 to 5 by 1.5 to 2, mostly 3.5 to 4.5 by 1.5 to 2 μ, pale yellowish in mass under the microscope; old spore tendrils coral red.

PERITHECIA.—Stromata the same or similar to the pycnidial stromata; perithecia dark, membranous, globose to flask shaped, collapsing when dry, 5 to 50 or sometimes more in a stroma, 300 to 400 μ in diameter, irregularly arranged in one to three layers and bearing slender necks projecting above the stroma, 300 to 600 μ, colored like the stroma on the outside and terminating in acute ostioles; asci oblong elliptical to subclavate, nearly sessile, 30 to 60 by 7 to 9 μ, mostly 40 to 50 by 8 μ; ascospores irregularly biseriate, ellipsoid, obtuse, sometimes constricted at the septum, hyaline, with a gelatinous envelope, 7 to 11 by 3.5 to 5 μ, mostly 8 to 9 by 4 to 4.5 μ.

CULTURAL CHARACTERS.—Cultures one month old on white corn meal have a white to pale orange yellow surface mycelium and produce numerous minute

pycnidia and pale yellow spore masses. It is distinguished from its nearest relative, *E. tropicalis*, by the lighter color of the mycelium.

HOSTS.—*Castanea dentata*, *C. sativa* and cult. vars., *C. pumila*, *Castanea mollissima* from China and *Castanea japonica* from Japan, *Quercus alba*, *Q. prinus*, *Q. velutina*, Acer sp.

It is also reported on *Rhus typhina* and *Carya ovata* by Anderson and Rankin.

TYPE LOCALITY.—Bronx Park, New York City.

GEOGRAPHICAL DISTRIBUTION.—Southern Maine to Ohio and southward to North Carolina; also Missouri, Iowa, Nebraska, British Columbia, China, and Japan.

ILLUSTRATIONS.—Murrill, 1908, *in* Torreya, v. 8, no. 5, p. 111, fig. 2; Petri, 1913, *in* Atti R. Accad. Lincei, Rend. Cl. Sci. Fis., Mat. e Nat., s. 5, v. 22; sem. 1, fasc. 9, p. 656, fig. 4; Heald, 1913, *in* Penn. Chestnut Tree Blight Com. Bul. 5, pl. 13; Clint. 1913, *in* Conn. Agr. Exp. Sta. Rpt., 1911/12, pl. 28, fig. c, f, i, and k; P. J. and H. W. And., 1913, *in* Penn. Chestnut Tree Blight Com. Bul. 4, p. 22, fig. 2, B and D; P. J. And. and Rank., 1914, *in* N. Y. Cornell Agr. Exp. Sta. Bul. 347, p. 562, fig. 89.

EXSICCATI.—Pycnidia and perithecia: Rehm, Asc., 1710; Wilson and Seaver, Asc. and Low. Fun., 3; Bart. Fun. Col., 2926; all on *Castanea dentata*.

This species is closely related in its morphological characters to all the species of section 2 of the genus. It is most likely to be confused with *E. fluens*, but shows constant differences, though slight, in size and shape of ascospores. They are predominantly broader and more uniform in shape, as shown by the table of measurements on page 35. In its active parasitic condition on Castanea it can always be distinguished by the presence of the mycelial "fans" in the inner bark, as shown in Plate VIII. It has been confused with *E. gyrosa* through an erroneous identification of that species.

## MORPHOLOGY AND DEVELOPMENT.

### MYCELIUM.

By far the most striking mycelial character is the production by *E. parasitica* of yellow or buff fan-shaped formations of mycelium in the cambium and bark of the host. These "fans" vary from 1 mm. to 1 cm. or more in width, and are composed of radiating hyphae closely pressed together to form a continuous layer. (Pl. VIII.) So constant are these mycelial fans in their occurrence and so characteristic in their appearance that they furnish the most reliable field character for distinguishing *E. parasitica* from related species and may quite properly be regarded as a specific character when the fungus is growing in living trees.

Anderson and Anderson (2, p. 204) first called attention to the fact that these fan-shaped formations of mycelium are absent from *E. fluens*. Rankin (62, p. 248) states that when the fungus grows saprophytically or while the tree is dormant these fans are not produced. Anderson and Rankin (6, p. 565) report that in inoculations

on *Quercus alba* and *Q. prinus*, *E. parasitica* produced the typical mycelial fans.

Anderson (1, p. 14) considers that the occurrence of these fans is associated with the parasitic habit of the fungus. In his opinion single hyphæ do not possess the power of penetrating the living cells, but the fungus grows on the injured and dead cells about a wound until a quantity of mycelium is accumulated, when it "*en masse* pushes through the living tissues of the bark." This view is also held by Keefer (45, p. 193), who adds that "the action of the advancing mycelial mats seems to be physical rather than chemical, and the cells are mechanically broken to pieces."

Rankin, however, states (62, p. 248) that "The host cells, just in advance of the edges of the fan, are disintegrated and form a distinct gelatinous band, which can be seen with the naked eye." This observation suggests to the writers that some toxic or enzymatic action upon the cells of the host probably occurs before the cells are actually invaded by the fungus hyphæ. Careful investigation of this point should go far toward determining the causes of the parasitism of this fungus. Whatever the cause or function of these fans, they are very characteristic, and the writers have found them invariably in diseased material of Castanea in America, as well as in that from China and in two specimens of *E. parasitica* on Quercus.

A similar mycelial formation, fanlike in form,[1] is produced by *Armillaria mellea* in the bark of roots attacked by this fungus. Excellent specimens of the Armillaria mycelial fans have been presented to the writers by Prof. Wm. T. Horne, of the University of California.

## STROMATA.

Under the name *Melogramma gyrosum*, in which they included specimens of both *Endothia gyrosa* and *E. fluens*, the Tulasnes (83, pp. 87–89) described the structure of Endothia in some detail. Their description was based chiefly on abundant local material of *E. fluens* collected on *Carpinus betulus* L. during several years, but they also used material sent by Guepin from western France, pycnidial material on chestnut from Italy, American material sent by Schweinitz to Brongniart and preserved in the Paris Museum, and specimens from Carolina sent by Berkeley. According to the Tulasnes (83, p. 87)[2] the stromata are "developed singly and emerge gradually as so many scattered points with fibers radiating in all directions, soon swell into a yellowish cone, rupture the epidermis above them,

[1] Since this manuscript was completed a very similar mycelial formation has come to the writers' attention. As figured by Nowell (50), pl. 1, *Rosellinia pepo*, when growing under the bark of lime trees, forms mycelial fans resembling those of *Endothia parasitica*.

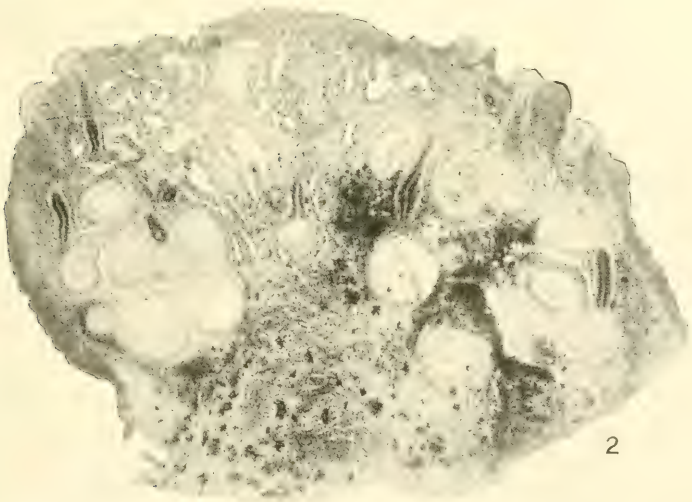
[2] The portions in quotations are rather free translations of the authors' Latin.

and put forth a very blunt apex. All are composed of a corky, parenchymatous, very dense, soft yellow material. The mature ones attain a diameter of 3 to 4 millimeters and a height of 1 to 2 millimeters, and on the somewhat reddish, and finally rusty red to yellow top, they are marked by black points, the ostioles." The Tulasnes observed that before the stromata reached their full size the pycnidial cavities were formed within them, sometimes "widely open," sometimes "narrow labyrinthine," and that through one or many openings in the top of the pycnidia, the long, twisted, orange tendrils, composed of mucus, and innumerable thin linear spores were expelled. "Perithecia are developed chiefly in stromata destitute of spermogonia, or more often with only a few * * * they arise very abundantly and irregularly, some barely buried in the yellow corklike substance, others lower down and seemingly located in the bark of the host itself."

Although the Tulasnes included all their material under a single species, they noted that the pycnidial stromata of the American specimens (really *Endothia gyrosa*) differed considerably from the European (*E. fluens*). In describing the former, they say (83, p. 88) "The American fungus is said to grow in the bark of Fagus and Juglans * * * as a whole it abounds with numerous, very small spermatia. Wherefore if it is very thinly sectioned, the pieces, examined with a compound microscope, show cavities just as if you had before your eyes the smallest Gautieria or Balsamia." The Tulasnes do not try to distinguish definitely between stroma and mycelium, but merely state that the stromata develop within the mycelium.

Ruhland (67), who was the next writer to discuss the morphology of a species of Endothia, defines the various portions of the fungus body in detail. According to his definition (p. 16) a "stroma (in distinction from mycelium) is the sum total of that part of the vegetative portion of the fungus body, which, without serving exclusively for absorption, takes part in the formation of the fruit body." He sets aside Fuisting's (36, p. 185) division of the fungus body into an epistroma and a hypostroma, as essentially nothing but the distinction of "conidial layers" and "perithecial stroma."

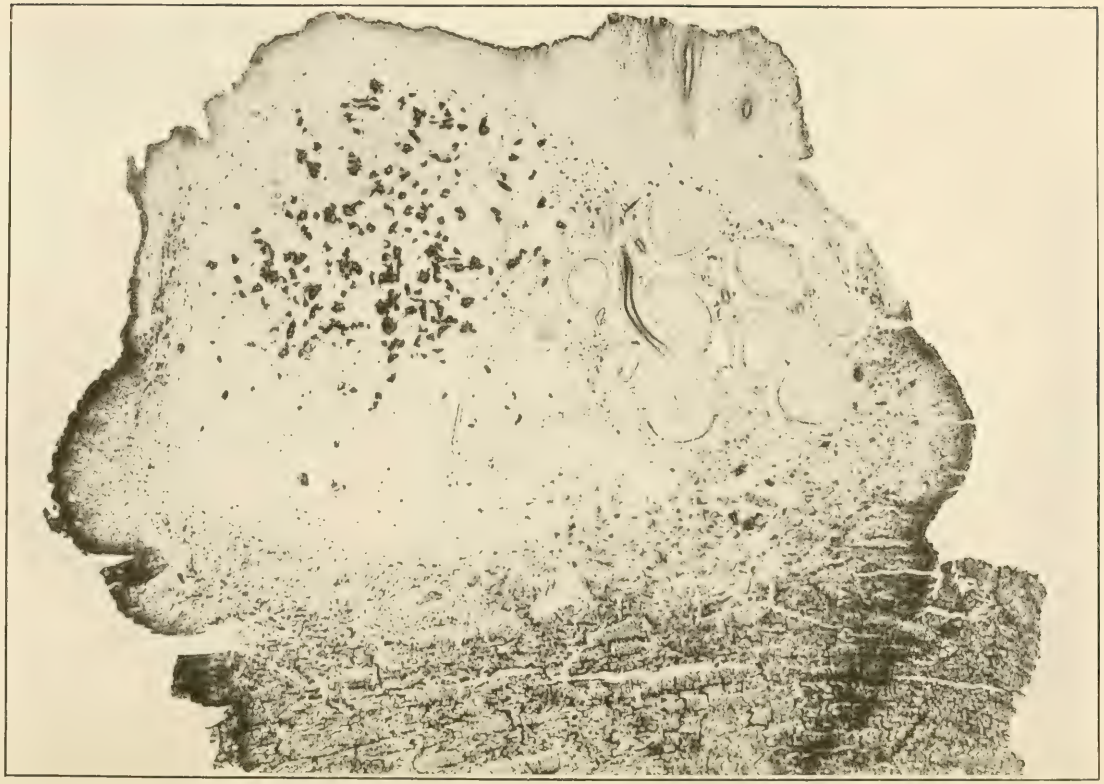
Ruhland divides the fungus body into an ectostroma and an entostroma. The ectostroma grows "on the upper surface of the parenchyma of the bark, between it and the periderm, and is composed of a generally wide-lumened plectenchyma which does not possess the power of absorption." This portion has the following functions: "The formation of the conidia, the opening and breaking off of the periderm, and the stimulation of the development of the entostroma." The entostroma, on the other hand, according to Ruhland, "lives in the parenchyma of the bark, and while young is in a high degree




ENDOTHIA GYROSA. VERTICAL SECTIONS OF STROMATA ON BEECH. × 32.

FIG. 1.—SHOWING NUMEROUS PYCNIDIAL CAVITIES AND TWO MATURE PERITHECIA.
FIG. 2.—SHOWING MATURE PYCNIDIA AND PERITHECIA SIDE BY SIDE.

Except where otherwise indicated, the photomicrographs of stromata are from unstained sections cut with a freezing microtome.



ENDOTHIA SINGULARIS. VERTICAL SECTION OF A STROMA, SHOWING BOTH PYCNIDIA AND PERITHECIA. × 32.

Paraffin section stained with Bismarck brown.

capable of absorption, a power which it retains relatively permanently." In addition to its absorptive function the entostroma forms the pseudoparenchymatic cover for the perithecial walls.

Ruhland studied herbarium material from the Royal Botanic Museum of Berlin and specimens from Saccardo and Cesati, and described it under the name of *Endothia radicalis* (Schw.) Fr. (*E. fluens* of the present writers). He distinguishes an ectostroma, shaped like a truncated cone, consisting of fine, thin-walled hyphæ, so closely interwoven that the whole structure has a comparatively firm quality. Among these hyphæ are crystals of calcium oxalate. As soon as this ectostroma breaks through the bark there is formed near the middle a short-lived 1-chambered pycnidium. Below this ectostroma (height 0.5 to 0.6 mm., diameter 0.7 to 1 mm.) the entostroma grows out as a mycelium through the upper portion of the bark. Ruhland says, "The entostroma with us does not produce perithecia, but remains wholly mycelial." He studied the perithecial stage in Cesati's specimens, however, and concludes that the perithecia originate without much change in the size of the entostroma and at a considerable distance, about 1 mm., below the ectostroma. The long necks then penetrate through the overlying entostroma and into the ectostroma to the base of the now functionless pycnidia. The upper portion of the ectostroma is then quickly killed and thrown off.

Pantanelli briefly described the stromata of the genus Endothia, and pointed out several morphological characters which he considers distinctive of *E. parasitica* in contrast to *E. fluens*. Aside from spore characters, which will be discussed later, Pantanelli (60, p. 870) considers that *E. parasitica* is characterized by numerous stromata, at first embedded in the bark, finally free; by pycnidial cavities numerous and irregularly arranged in various planes in the stromata deep in the bark; pycnidial stromata 1.1 to 1.2 mm. in height and 2.1 to 2.2 mm. in diameter; ascogenous stromata, height 1.8 to 2 mm., length 2.5 to 3.4; width, 3 to 3.2 mm.; perithecia arranged in two or three layers; necks of perithecia averaging 1.25 mm., with inconspicuous ostioles; walls of the perithecia uncolored or light brown.

*Endothia fluens*, on the other hand, has isolated stromata, chiefly outside the bark; pycnidia aggregated, regularly arranged in a single superficial series; pycnidial stromata, height 0.4 to 0.5 mm., diameter 1.1 to 1.3 mm.; ascogenous stromata, height 1.1 to 1.4 mm., length 2.5 to 3.2 mm., width 1.2 mm.; perithecia arranged in a single row; necks of perithecia averaging 0.45 mm.; ostioles prominent; walls of the perithecia black.

Anderson (1, pp. 17–24) described the development of the fructifications of *Endothia parasitica* in detail. He studied the growth of

the pycnidia in pure culture and made sections of perithecial stromata growing on bark.

According to Anderson, the pycnidium originates as a mass of densely intertwined hyphæ, in the center of which numerous pycnospores are cut off. The crowding of these spores increases the size of the pycnidial cavity and crowds the outer hyphæ together to form a sort of wall. The ostiole is formed in the top by the loosening of the hyphæ. The stroma always starts as a loose growth of hyphæ around the pycnidium. It does not precede, but follows the first stages in the development of that organ. A fluffy growth of light-yellow mycelium surrounds the pycnidium and covers it over. If these are embedded and sectioned, they will be found to contain a loose tangle of undifferentiated hyphæ surrounding a central pycnidium. But as soon as the cork layer is broken the stroma undergoes a change. There is a rapid increase in size and at the same time a differentiation of the cells at the tips of those branches which reach the exposed surface. These cells now become shorter and thicker, acquire heavier walls, and are densely crowded together, so that in cross section they appear as a pseudoparenchymatous tissue. The layer thus formed covers all the exposed surface of the stroma and also grows up around the necks of the perithecia. The stroma increases very rapidly in size and a mass of stromatic tissue is formed beneath the pycnidia, which are thus pushed out through the cork layer into the periphery. The primordia of the perithecia are formed usually in the tissues of the bark below the base of the original pycnidium, but at times are formed well up in the stroma. Usually 15 to 30 perithecia mature in a stroma.

According to the writers' observations, the Tulasnes' description (83, pp. 87–89) is substantially correct so far as it goes. They, of course, placed pycnidial material of *Endothia gyrosa* in the same species with *E. fluens*, but, as already noted, they observed the difference in the structure of the stromata and aptly compared the pycnidial stroma of *E. gyrosa*, as seen in section, to a Gautieria.

The division of the stroma into ectostroma and entostroma made by Ruhland (67, p. 16) has, at least in the species of Endothia, no validity whatever. While it is true that pycnidia usually occur in the portion of the stroma first developed and perithecia often develop below them, this is by no means an invariable rule; and while stromata are developed which contain only pycnidia, other stromata apparently produce only perithecia or no spores whatever. Certainly no portion of the stroma can be distinguished which invariably produces only perithecia or only pycnidia. On the contrary, there is great variation in the relative position and time of appearance of the two types of fruiting structures. Also, while

the pycnidial cavity is sometimes small and simple, as described by Ruhland, it is more often large and much convoluted. (See Pls. XV and XVI.)

While the writers, of course, agree with Pantanelli (60) that *Endothia parasitica* and *E. fluens* are distinct species, many of the stromatic characters which he describes are so variable as to be unreliable. In an examination of a large number of specimens the writers have been unable to find any constant difference in the arrangement or structure of the pycnidial stromata. This seems to depend chiefly in both species on the character of the bark and the moisture conditions. As to size, while the stromata of *E. parasitica* examined average somewhat larger than those of *E. fluens*, the range of the pycnidial stromata is about the same in the two species, varying from 0.4 to 2 mm. in height and from 0.2 to 3 mm. in length.

The ascogenous stromata are also very variable in size. Those measured by the writers varied in height from 0.5 to 2 mm. in *Endothia parasitica* and from 0.5 to 2.3 mm. in *E. fluens*. In width the perithecial stromata were from 1 to 2.5 mm. in both species, while there is apparently no method for determining their length, since on thick-barked trees continuous narrow masses of perithecial stromata are often formed in the crevices of the bark. These stromatal masses frequently extend from 5 to 10 cm., and while they are in all probability formed by the fusion of several stromata there is no way of determining how far each extends.

The arrangement of the perithecia mentioned by Pantanelli (60) as a specific character seems to depend on the nature of the bark of the host. When the bark is thin and easily ruptured the stromata tend to spread out so that the perithecia occur in a single layer, while if the bark is thick and deeply ridged the stromata are thicker and the perithecia occur in two or more layers. That this is not a specific character is clearly shown by Plate XVI. Figures 1 and 3 of this plate show a stroma of *E. parasitica* and of *E. fluens*, respectively, both with three layers of perithecia, while Plate XVI, figure 2, and Plate X, figure 1, show stromata of both species with perithecia arranged in a single layer.

Although, as already indicated, the stromata of each species are very variable, they are sufficiently distinct so that the native American species may readily be distinguished in the field.

The stromatic characters of *Endothia gyrosa* and *E. singularis* are much more distinct than those of the other species. The stromata of *E. gyrosa* are erumpent, irregularly subglobose, with a rather roughened surface. They are usually from 1.5 to 2 mm. in height and vary from 1.5 to 3 mm. in width. The stromata of *E. singularis* are much larger than those of any other species of Endothia, being

usually from 2 to 4 mm. in length and 3 to 5 mm. or more in diameter. They are decidedly erumpent, rather regular, and subglobose in outline. The contents of the stromata are brick red in color and are very powdery when old.

The stromata of *Endothia fluens*, *E. fluens mississippiensis*, and *E. parasitica* resemble each other so closely that the species are practically indistinguishable on this basis. All these species are characterized by partially embedded, confluent stromata which vary greatly in outline, depending on the nature of the bark of the host. As already stated, they vary from 0.4 to 2 mm. in height and from 0.7 to 5 mm. or more in length where confluent. *E. tropicalis* and *E. longirostris* resemble this group in their stromatic characters.

*Pycnidia.*—The pycnidia of *Endothia gyrosa* and *E. singularis* are very distinctive also. The pycnidial cavities of *E. gyrosa* are narrow and so irregularly convoluted that in a section of the stroma the cavities vary in width from 0.03 to 0.3 mm., averaging about 0.15 mm. On the whole, however, they are much narrower than those of *E. fluens* or *E. parasitica*. A section of a pycnidial stroma of *E. gyrosa* shows numerous irregular, rounded to elongate chambers separated by narrow walls. The pycnidial cavities of *E. singularis* (Pl. XIII) are minute, 0.03 mm. in diameter, nearly spherical, evenly distributed through the stroma and separated at first by comparatively thick walls, which disintegrate and become powdery when the stroma is old.

So far as the writers have been able to determine, the "tendrils" of pycnospores so characteristic of *Endothia fluens* and *E. parasitica* are not formed in either *E. gyrosa* or *E. singularis*. Mature pycnidial stromata of *E. gyrosa* when placed in a moist chamber exude numerous droplets containing spores and scattered well over the surface of the stromata. The writers have been unable to produce any such change by placing the pycnidial stromata of *E. singularis* in moist chambers, and it seems probable that the pycnospores of *E. singularis* are set free by the breaking down of the outer walls of the stromata. As already mentioned, the inner partitions are friable, so the spores are readily scattered by the wind.

The pycnidial cavities of *Endothia fluens* and *E. parasitica*, and apparently all the other species of this section of the genus, vary from 0.2 to 0.3 mm. or more in diameter and may consist of a single chamber rather regular in outline (Pl. XIV, fig. 1) or of an irregular cavity consisting of many chambers (Pl. XV, fig. 3) more or less completely separated from one another. These species differ from *E. gyrosa* in that the pycnospores are usually discharged through a single opening near the top of the stroma and emerge in a single twisted tendril.

*Development of the stromata.*—The writers have not followed the development of the stromata in culture, but an examination of numerous sections of *Endothia singularis*, *E. gyrosa*, *E. fluens*, and *E. parasitica* and a study of the three latter species under field conditions on various hosts shows that their development is by no means as uniform as indicated in Anderson's description (1).

According to Anderson, the pycnidium develops first, and about the young pycnidium the stroma is quickly formed, while the perithecia arise later, usually in the lower portion of the stroma. This may perhaps be considered the typical course of development, and pycnidia are often found above the perithecia, but all variations occur. A large stroma may be developed without a sign of a pycnidium (Pl. XV, fig. 2). In some cases there is a considerable portion of the stroma above the pycnidial cavity (Pl. XIV, fig. 2), or the pycnidial cavities may be surrounded by a thick stroma (Pl. XIV, fig. 4, and Pl. XV, fig. 1). Sometimes, on the other hand, they are large and irregular, with little stroma (Pl. XV, fig. 3).

The perithecia by no means uniformly arise below the pycnidia, but the two often occur side by side in the same stroma (Pl. IX, fig. 2; Pl. XIV, fig. 3; and Pl. XII). Sometimes, even, the perithecia are above the pycnidia (Pl. XIV, fig. 2). There seems to be no constant relation either as to the relative number of pycnidia or of perithecia in a single stroma. Sometimes the pycnidial portion is much larger (Pl. IX, fig. 1); sometimes the perithecia predominate (Pl. X, fig. 2); and sometimes the two portions are practically equal (Pl. XII).

A like variability apparently occurs in the sequence of the fruiting bodies. As the figures show, the pycnidia sometimes develop after the perithecia; the reverse order is frequent; while in several sections (Pl. XII, and Pl. XIV, fig. 3) the two types of fruiting bodies were side by side and were producing mature spores abundantly at the same time. Just what factors determine the production of each type of spore or prevent or delay spore production is unknown. It seems probable, however, that climatic influences may prevent the development of ascospores in many cases. The action of climate may be very indirect, however, for no ascospores of any species have yet been obtained in artificial cultures, though *Endothia fluens*, *E. fluens mississippiensis*, *E. tropicalis*, and *E. parasitica* produce pycnospores abundantly on a variety of media. Certainly, climatic factors would not account satisfactorily for the fact that pycnidia and perithecia are produced at the same time in adjacent stromata, or even in different parts of the same stroma.

The size of the perithecia is rather uniform in the various species (Pl. X, fig. 3, and Pls. XI and XVI), being about 0.35 mm. in diameter. They are typically globose to pyriform, but are usually more or

less irregular on account of crowding. This pressure may be so great as to produce almost any shape, and such perithecia sometimes measure 0.5 mm. in the greatest diameter and 0.1 mm. in the shortest.

## SPORE MEASUREMENTS.

The spore measurements recorded here were made by Miss Tiller. In the case of dried specimens, the spores were first soaked for three hours in lukewarm water and then mounted in the potassium-glycerine-copper medium, prepared according to the following formula:

1 part 2 per cent potassium acetate in water.
1 part 40 per cent glycerine in alcohol.
Copper acetate sufficient to color.

In the case of fresh specimens they were mounted directly in the same medium. The measurements were made with a Zeiss filar eyepiece micrometer and a Zeiss 3 mm. 1.40 N. Ap. oil-immersion objective. Only approximate accuracy is claimed for these results, on account of the difficulty of overcoming the motion of the spores in a fluid medium. The results are, however, believed to be fairly comparable, as practically all were measured under the same conditions and treatment, and the margin of error is presumably rather uniform. The differences in size of pycnospores do not appear to be sufficient, however, to furnish diagnostic character for most of the species.

The number of measurements of ascospores of *Endothia fluens* and *E. parasitica* is much larger than of the other species, as special attention was first given to these two species on account of their great similarity. In order to make the measurements of these species comparable to the others, the total number of spores of each length has been calculated in the percentage of the total number of spores measured.

### METHOD OF TABULATION.

For better comparison, the spore measurements have been tabulated by half microns, all the spores in each specimen coming within 0.2 of a micron of each unit or half being grouped together; e. g., all the spores having a length of 7.3, 7.4, 7.5, 7.6, and 7.7 microns are included under the heading 7.5. The tables thus show at a glance the number of spores of a given length per specimen. The widths have been tabulated in the same way.

For a better comparison of the shapes of the ascospores of *Endothia parasitica* and *E. fluens*, the relative ratios of length to width in each spore have been calculated, the width being considered unity. The ratios of length to width were then tabulated by tenths; that is, all the spores in each specimen having a ratio of length to

width from 1.76 to 1.85 microns are included under 1.8. The relative shapes of the spores in each specimen are thus clearly shown.

TABLE I.—*Measurements of pycnospores and asci of Endothia.*

PYCNOSPORE MEASUREMENTS.

| Specimens. | Number per specimen having the given length or width. | | | | | | | | | | | | | | | Total. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Lengths (microns). | | | | | | | | | | | Widths (microns). | | | | |
| | 2 | 2.5 | 3 | 3.5 | 4 | 4.5 | 5 | 5.5 | 6 | 6.5 | 7 | 1 | 1.5 | 2 | 2.5 | |
| Sphaeria gyrosa Schw., Herb. Mus., Paris | 2 | 3 | 6 | **11** | 4 | .... | .... | .... | .... | .... | .... | 9 | **15** | 2 | .... | 26 |
| S. gyrosa Schw., Herb. Schwaeg. | .... | .... | 5 | **9** | 3 | .... | .... | .... | .... | .... | .... | 5 | **11** | 1 | .... | 17 |
| Endothia gyrosa on beech, Alcorn, Miss., No. 1796 | .... | .... | 10 | **11** | 3 | .... | .... | .... | .... | .... | .... | 5 | **18** | 1 | .... | 24 |
| E. singularis, No. 1939 | .... | .... | 4 | **6** | 4 | .... | .... | .... | .... | .... | .... | .... | 5 | **9** | .... | 14 |
| E. fluens, Sowerby | .... | .... | .... | 8 | **11** | 6 | .... | .... | .... | .... | .... | .... | 8 | **17** | .... | 25 |
| E. fluens on chestnut, Fort Payne, Ala | .... | .... | 1 | 6 | **12** | 5 | 1 | .... | .... | .... | .... | .... | 6 | **19** | .... | 25 |
| E. fluens, Lugano, Switzerland | .... | .... | 1 | 3 | **5** | 2 | 1 | .... | .... | .... | .... | .... | 8 | 4 | .... | 12 |
| E. fluens mississippiensis, No. 1706A | .... | .... | 2 | 5 | **8** | 1 | .... | .... | .... | .... | .... | .... | 7 | **9** | .... | 16 |
| E. longirostris | 2 | 3 | **4** | **4** | 1 | .... | .... | .... | .... | .... | .... | 6 | **8** | .... | .... | 14 |
| E. tropicalis | .... | .... | .... | 2 | 5 | **6** | 3 | 3 | 2 | .... | 4 | .... | 3 | **19** | 3 | 25 |
| E. parasitica, No. 1696 | .... | .... | 1 | 6 | **11** | 6 | 1 | .... | .... | .... | .... | .... | 12 | **13** | .... | 25 |

LENGTHS OF ASCI (MICRONS).

| Specimens. | No. | Number per specimen having the given length. | | | | | | | | Total. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 | |
| Endothia fluens on Castanea | 1702 | 2 | 8 | **11** | 1 | ..... | ..... | ..... | ..... | 22 |
| Do | 1737 | ..... | 1 | **14** | 11 | ..... | ..... | ..... | ..... | 26 |
| Do | 1741 | ..... | 5 | **6** | ..... | ..... | ..... | ..... | ..... | 11 |
| Do | 1729 | ..... | 4 | **16** | 6 | ..... | ..... | ..... | ..... | 26 |
| E. fluens on dead Castanea | 1715 | ..... | 7 | **16** | 3 | ..... | ..... | ..... | ..... | 26 |
| E. fluens on Castanea, Stresa, Italy | 1656 | ..... | 6 | **16** | 1 | 1 | 1 | ..... | ..... | 25 |
| E. fluens on Quercus | 1711 | 2 | **16** | 7 | ..... | ..... | ..... | ..... | ..... | 25 |
| Do | 1927 | 2 | 10 | **12** | ..... | ..... | ..... | ..... | ..... | 24 |
| E. fluens mississippiensis, Blue Mountain, Miss | 1806 | ..... | 5 | **14** | 3 | ..... | ..... | ..... | ..... | 22 |
| E. parasitica on Castanea | 1710 | ..... | 1 | ..... | 1 | **2** | ..... | ..... | ..... | 4 |
| Do | 1739 | ..... | ..... | 5 | **10** | **10** | 1 | ..... | ..... | 26 |
| E. parasitica, China | 2151 | ..... | ..... | ..... | 2 | **14** | 4 | 4 | 3 | 27 |
| E. gyrosa on Quercus | 1709 | 1 | **7** | ..... | ..... | ..... | ..... | ..... | ..... | 8 |
| E. gyrosa on Liquidambar | ..... | **19** | 6 | ..... | ..... | ..... | ..... | ..... | ..... | 25 |
| E. singularis, Palmer Lake, Colo | ..... | 4 | **15** | 5 | ..... | ..... | ..... | ..... | ..... | 24 |
| E. longirostris, Porto Rico | ..... | 6 | **13** | 3 | 3 | ..... | ..... | ..... | ..... | 25 |
| E. longirostris, French Guiana | ..... | 6 | **10** | 1 | ..... | ..... | ..... | ..... | ..... | 17 |
| E. tropicalis | ..... | ..... | ..... | ..... | ..... | **6** | 5 | 3 | 2 | 16 |

WIDTHS OF ASCI (MICRONS).

| Specimens. | Number per specimen having the given width. | | | | | | | Total. |
|---|---|---|---|---|---|---|---|---|
| | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| Endothia gyrosa | ..... | ..... | **8** | 2 | ..... | ..... | ..... | 10 |
| E. singularis | 1 | **8** | 2 | ..... | ..... | ..... | ..... | 11 |
| E. fluens, Europe | ..... | ..... | 2 | **6** | 4 | ..... | ..... | 12 |
| E. fluens, America | ..... | ..... | 1 | **6** | 4 | ..... | ..... | 11 |
| E. fluens mississippiensis | ..... | ..... | 2 | **6** | 2 | ..... | ..... | 10 |
| E. longirostris, Porto Rico | ..... | 2 | **7** | 2 | ..... | ..... | ..... | 11 |
| E. longirostris, French Guiana | ..... | ..... | 3 | 3 | 3 | ..... | ..... | 9 |
| E. tropicalis | ..... | ..... | ..... | 2 | **6** | 2 | ..... | 10 |
| E. parasitica, China | ..... | ..... | ..... | 1 | **7** | 2 | ..... | 10 |
| E. parasitica, America | ..... | ..... | ..... | ..... | **6** | 4 | ..... | 10 |

### PYCNOSPORES.

The pycnospores of all the species are oblong elliptic to cylindric in shape and so small as to make accurate measurement very difficult. Slight but apparently constant differences in their size in certain groups of species may, however, be traced. These differences are clearly shown in Table I.

*Endothia gyrosa*, *E. singularis*, and *E. longirostris* have smaller pycnospores than the other species, the most frequent lengths being 3 and 3.5 μ. The pycnospores of *E. singularis* are slightly broader than those of *E. gyrosa* and *E. longirostris*, being 1.5 to 2 μ, as against 1 to 1.5 μ in the last two species.

*Endothia fluens*, *E. fluens mississippiensis*, and *E. parasitica* are even more closely similar in the size of their pycnospores than in that of their ascospores, the most frequent size being 4 by 2 μ. The pycnospores of *E. tropicalis* are much larger and more variable in size and shape than those of other species. They range from 3.5 to 7 μ in length and from 1.5 to 2.5 μ in width.

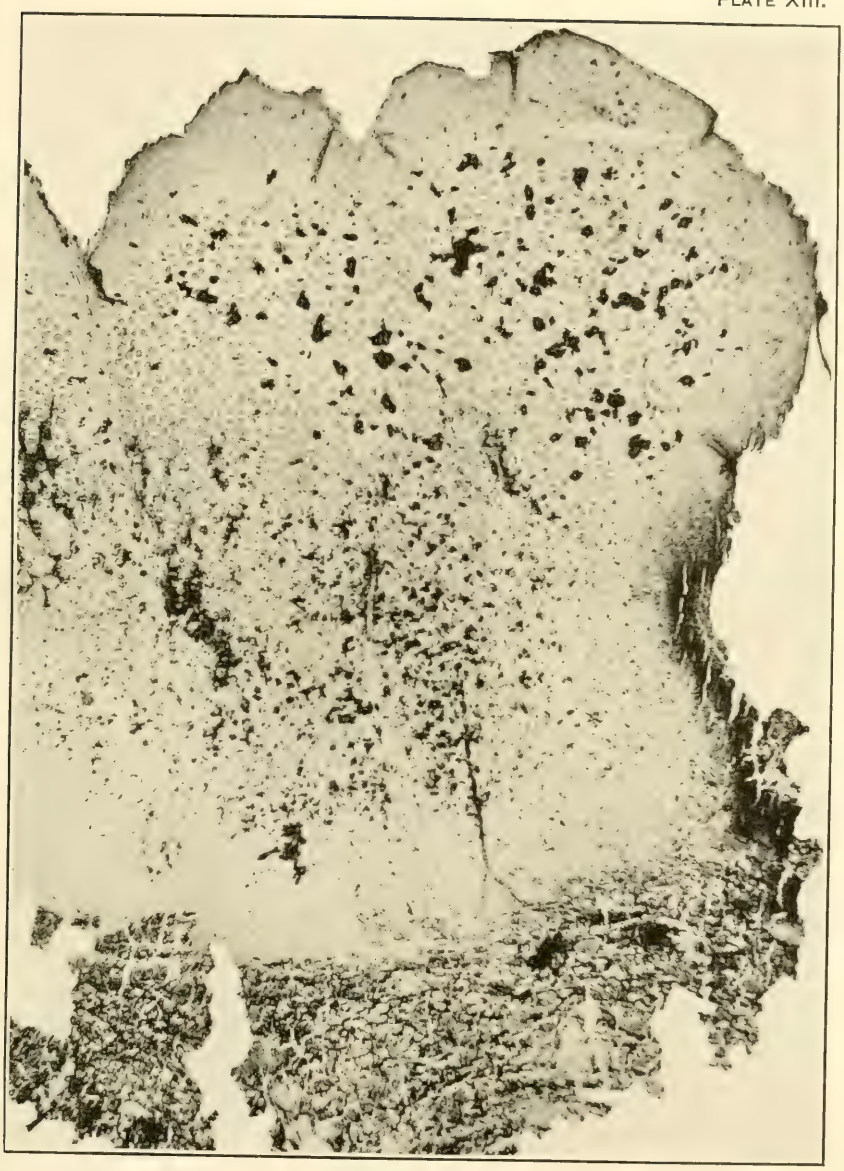
### ASCI.

The writers have not attempted a study of the origin and early development of perithecia or asci in any of the species of Endothia. Work on this subject has been published by Anderson and Rankin (6), for *Endothia parasitica*, but the nuclear phenomena and origin and development of the ascogenous hyphæ are not yet entirely clear. The part termed a trichogyne by these authors seems more likely to be the initial stage in the development of the neck of the perithecium than the relic of an organ of fertilization.

The asci appear almost or quite sessile in most species, and though varying considerably in size and shape, as indicated in Table I, are usually oblong elliptic or subclavate, having a sort of inner membrane inclosing the ascospores and some thin granular matter extending to the apex of the ascus, where a slight thickening appears, as described and illustrated by Anderson for *Endothia parasitica*. A similar condition is found in various species of Pyrenomycetes and probably functions in some way in connection with the discharge of the ascospores. The asci are generally wider and slightly longer in *E. parasitica* than in *E. fluens* and other members of section 2. The asci of *E. gyrosa* are shorter than those of any other species. *E. tropicalis* has the longest asci. The asci of none of the species show a very wide range of variation, as Table I also indicates.

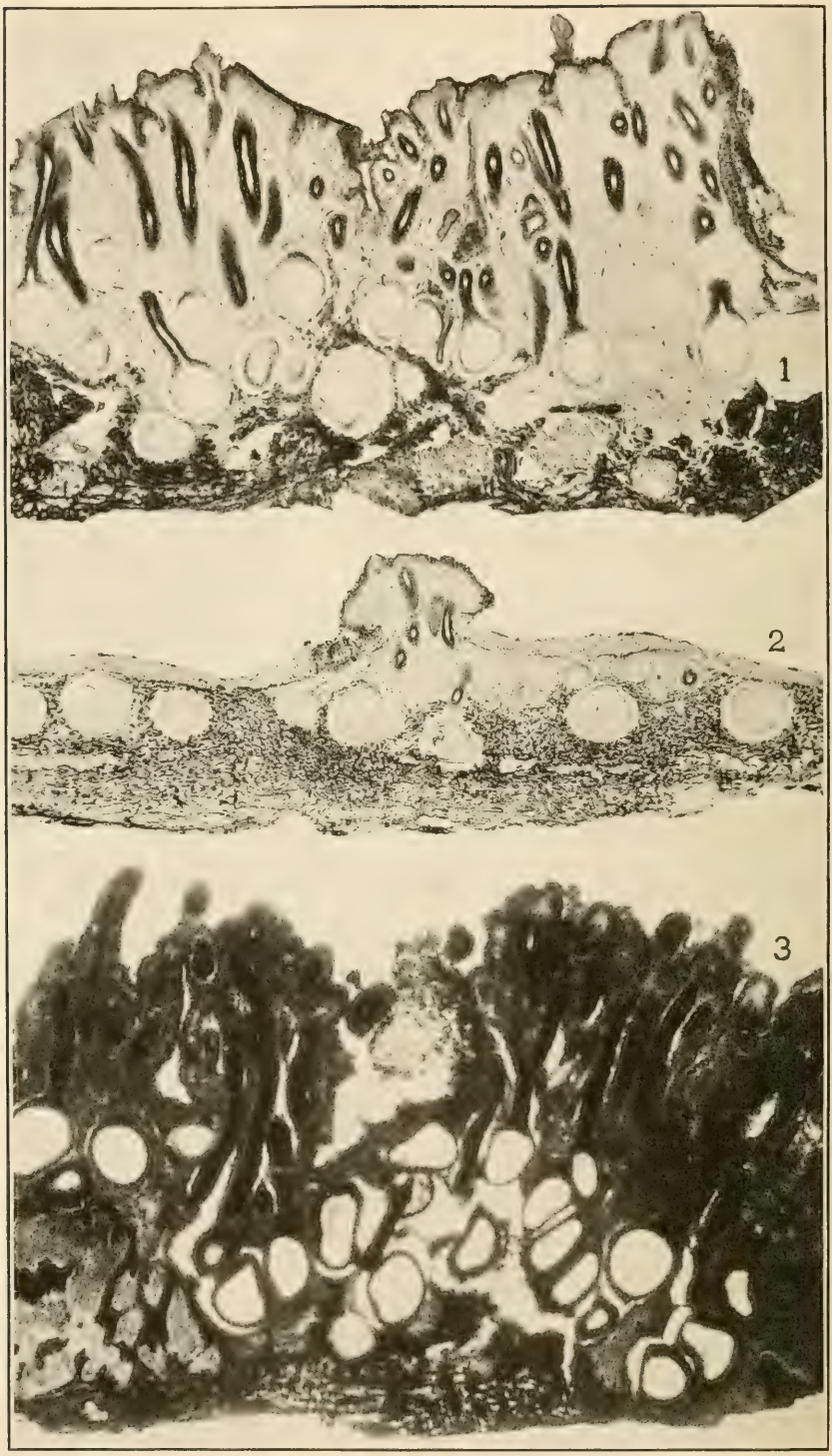
### PARAPHYSES.

Most students of Endothia have reported paraphyses wanting in this genus. Anderson (1, p. 33, fig. 32) and Anderson and Rankin (6, p. 579, fig. 83) report paraphyses present and figure what they



ENDOTHIA SINGULARIS. VERTICAL SECTION OF THE MAJOR PART OF A PYCNIDIAL STROMA. × 32.

Paraffin section stained with Bismarck brown.




ENDOTHIA PARASITICA AND E. FLUENS. VERTICAL SECTIONS OF STROMATA. × 20.

FIG. 1.—E. PARASITICA. SHOWING PERITHECIA ARRANGED IN SEVERAL IRREGULAR LAYERS. FIG. 2.—E. PARASITICA, SHOWING PERITHECIA ARRANGED IN A SINGLE LAYER. FIG. 3.—E. FLUENS, FROM ITALY, SHOWING PERITHECIA ARRANGED IN SEVERAL LAYERS.

regard as an early stage of their development. They describe them as branching frequently and very crooked, extending around the perithecium as well as upward. The writers have searched in all the species studied for evidence of the presence of paraphyses, but have never seen anything resembling paraphyses as they occur in closely related Pyrenomycetes. If they occur, they would seem to be of an unusual character and difficult to recognize or else are evanescent, disappearing before the asci are mature.

### ASCOSPORES.

The ascospores furnish one of the most marked characters for the separation of the genus into sections (Plate XVII). In section 1 they are more or less cylindric and sometimes curved. In section 2 they are more or less elliptic, being broadest in *Endothia parasitica* and narrowest in *E. fluens* and *E. longirostris*. The greatest variation in size and shape of ascospores occurs in *E. fluens*, as indicated by the measurements given in Table II. Anderson (1), Clinton (18), and Heald (39) describe and figure the ascospores of *E. parasitica* as very obtuse and constricted at the septum. The writers have but rarely seen spores of this form. This may perhaps be due in part to different methods of treatment or to the age and condition of the material. Most of the ascospores studied by the writers have been mounted in the fluid medium described on page 30. Fresh specimens have also been studied in water mounts, but with the same general result. The writers are of the opinion, therefore, that the figures of the authors cited above do not represent the most common and characteristic form of ascospores of this species. (Compare Plate XVII, figs. 7 to 15.)

TABLE II.—*Measurements of ascospores of Endothia.*

| Specimens. | No. | Number per specimen having the given length or width. | | | | | | | | | | | | | | | | | | | | Total. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lengths (microns). | | | | | | | | | | | | Widths (microns). | | | | | | | | |
| | | 6 | 6.5 | 7 | 7.5 | 8 | 8.5 | 9 | 9.5 | 10 | 10.5 | 11 | 11.5 | 12 | 1.5 | 2 | 2.5 | 3 | 3.5 | 4 | 4.5 | 5 | |
| Endothia gyrosa on Liquidambar, Cyprus, Tenn. | 1797 | ..... | ..... | 1 | 15 | 13 | 22 | 22 | 11 | 7 | 5 | 4 | 1 | ..... | ..... | 57 | 42 | 2 | ..... | ..... | ..... | ..... | 101 |
| E. singularis on Quercus utahensis, Palmer Lake, Colo. | ..... | ..... | ..... | 3 | 10 | 9 | 23 | 27 | 11 | 11 | 2 | 2 | ..... | 1 | 6 | 77 | 15 | 1 | ..... | ..... | ..... | ..... | 99 |
| Sphaeria radicalis, Fries herbarium | ..... | 12 | 15 | 33 | 24 | 5 | 1 | 1 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 52 | 34 | 5 | ..... | ..... | 91 |
| Endothia fluens on Castanea dentata: | | | | | | | | | | | | | | | | | | | | | | | |
| Taylorsville, N. C. | 1729 | 5 | 6 | 12 | 25 | 18 | 18 | 8 | 3 | 2 | ..... | 1 | ..... | ..... | ..... | ..... | 1 | 23 | 43 | 28 | 3 | ..... | 98 |
| Connellsville, Pa. | 1702 | 12 | 18 | 19 | 24 | 13 | 9 | 3 | 1 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 40 | 57 | 2 | ..... | ..... | 99 |
| Forest, Va. | 1741 | 3 | 6 | 12 | 27 | 8 | 22 | 16 | 3 | ..... | ..... | 1 | ..... | ..... | ..... | ..... | 1 | 34 | 47 | 15 | 1 | ..... | 98 |
| Monticello, Va. | 1737 | ..... | 2 | 10 | 16 | 19 | 21 | 16 | 9 | 4 | 1 | ..... | 1 | ..... | ..... | ..... | ..... | 1 | 25 | 65 | 8 | ..... | 99 |
| E. fluens: | | | | | | | | | | | | | | | | | | | | | | | |
| On Quercus coccinea, Blacksburg, Va. | 1927 | ..... | 7 | 25 | 27 | 6 | 5 | 1 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 7 | 40 | 24 | ..... | ..... | 71 |
| From dead Castanea dentata, Asheville, N. C. | 1715 | 2 | 15 | 18 | 19 | 19 | 12 | 8 | 1 | ..... | ..... | 1 | ..... | ..... | ..... | ..... | ..... | 25 | 62 | 8 | ..... | ..... | 95 |
| On stump of Castanea vesca, Stresa, Italy | 1656 | ..... | ..... | 8 | 12 | 13 | 31 | 17 | 6 | 7 | 3 | 1 | 2 | 1 | ..... | ..... | ..... | 3 | 30 | 67 | 1 | ..... | 101 |
| On Castanea vesca, Como, Italy | 1637 | ..... | ..... | 10 | 18 | 18 | 24 | 16 | 8 | 2 | 1 | 1 | ..... | ..... | ..... | ..... | ..... | 42 | 47 | 9 | ..... | ..... | 98 |
| Do. | 1655 | 1 | 1 | 12 | 26 | 19 | 15 | 18 | 5 | 2 | 1 | ..... | ..... | ..... | ..... | ..... | ..... | 37 | 51 | 12 | ..... | ..... | 100 |
| Total | ..... | 23 | 55 | 126 | 194 | 133 | 157 | 103 | 36 | 17 | 6 | 5 | 3 | 1 | ..... | ..... | 2 | 212 | 402 | 230 | 13 | ..... | 859 |
| Per cent | ..... | 2.6 | 6.4 | 14.6 | 22.6 | 15.5 | 18.3 | 11.9 | 4.9 | 1.9 | 0.7 | 0.6 | 0.3 | 0.1 | ..... | ..... | 0.2 | 24.7 | 46.7 | 26.7 | 1.4 | ..... | ..... |
| E. pseudoradicalis | ..... | 4 | 13 | 28 | 27 | 16 | 11 | 1 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 29 | 57 | 14 | ..... | ..... | 100 |
| E. fluens mississippiensis: | | | | | | | | | | | | | | | | | | | | | | | |
| On Castanea dentata, Blue Mountain, Miss. | 1782 | ..... | 3 | 9 | 11 | 10 | 8 | 5 | 1 | 1 | ..... | 1 | ..... | ..... | ..... | ..... | ..... | 7 | 36 | 6 | ..... | ..... | 49 |
| Do. | 1806 | 4 | 2 | 18 | 13 | 5 | 8 | 1 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 20 | 27 | 4 | ..... | ..... | 51 |
| Total | ..... | 4 | 5 | 27 | 24 | 15 | 16 | 6 | 1 | 1 | ..... | 1 | ..... | ..... | ..... | ..... | ..... | 27 | 63 | 10 | ..... | ..... | 100 |
| E. longirostris: | | | | | | | | | | | | | | | | | | | | | | | |
| Porto Rico | ..... | 6 | 10 | 28 | 31 | 14 | 10 | 1 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 50 | 47 | 3 | ..... | ..... | 100 |
| French Guiana | ..... | 7 | 14 | 24 | 36 | 11 | 6 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 28 | 60 | 10 | ..... | ..... | 98 |

| Specimens. | No. | 1.6 | 1.7 | 1.8 | 1.9 | 2 | 2.1 | 2.2 | 2.3 | 2.4 | 2.5 | 2.6 | 2.7 | 2.8 | 2.9 | 3 | 3.1 | 3.2 | Total. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Number per specimen having the given ratio of lengths to widths. | | | | | | | | | | | | | | | | | |
| Sphaeria radicalis, Fries's herbarium | | | 1 | 3 | 6 | 10 | 17 | 19 | 9 | 10 | 14 | 2 | | | | | | | 91 |
| Endothia fluens: | | | | | | | | | | | | | | | | | | | |
| North Carolina | 1729 | | 2 | 3 | **16** | 8 | 8 | **16** | 15 | 8 | 6 | 4 | 3 | 5 | 2 | 1 | 1 | | 98 |
| Do | 1715 | | | 3 | 6 | 11 | 12 | 9 | 12 | **15** | 11 | 8 | 3 | 2 | 2 | 1 | | | 95 |
| Pennsylvania | 1702 | | 1 | 4 | 10 | 12 | 12 | **24** | 14 | 8 | 7 | 3 | 3 | | | | 1 | | 99 |
| Virginia | 1741 | | | 2 | 4 | 10 | 12 | 10 | 7 | 9 | **13** | **13** | 7 | 6 | 2 | 2 | 1 | | 98 |
| Do | 1927 | | 1 | 6 | **16** | **16** | 12 | 13 | 3 | 3 | 1 | | | | | | | | 71 |
| Do | 1737 | | | 4 | 15 | **18** | 16 | 7 | 14 | 11 | 6 | 3 | 4 | | | 1 | | | 99 |
| Total, American | | | 4 | 22 | 67 | 75 | 72 | 79 | 65 | 54 | 44 | 31 | 20 | 13 | 6 | 5 | 3 | | 560 |
| Per cent | | | 0.7 | 3.9 | 12.0 | 13.4 | 12.9 | 14.1 | 11.6 | 9.7 | 7.9 | 5.5 | 3.6 | 2.3 | 1.1 | 0.9 | 0.5 | | |
| Endothia fluens: | | | | | | | | | | | | | | | | | | | |
| Stresa, Italy | 1656 | | | 5 | 4 | 11 | **23** | 13 | 12 | 13 | 1 | 6 | 4 | 3 | 3 | 2 | | 1 | 101 |
| Como, Italy | 1637 | | | | | 1 | 7 | 8 | 12 | 10 | 11 | **20** | 12 | 9 | 4 | 2 | 2 | | 98 |
| Do | 1655 | | | | 2 | 3 | 11 | 12 | 12 | **17** | 12 | 13 | 7 | 5 | 2 | 1 | 2 | 1 | 100 |
| Total, European | | | | 5 | 6 | 15 | 31 | 33 | 36 | 40 | 24 | 39 | 23 | 17 | 9 | 5 | 4 | 2 | 299 |
| Per cent | | | | 1.7 | 2.0 | 5.0 | 13.8 | 11.1 | 12.1 | 13.4 | 8.0 | 13.1 | 7.7 | 5.7 | 3.0 | 1.7 | 1.4 | 0.7 | |
| Endothia parasitica: | | | | | | | | | | | | | | | | | | | |
| On Castanea sp., Santunying, China | 2151 | 2 | 11 | 17 | **33** | 22 | 9 | 3 | 2 | 1 | | | | | | | | | 100 |
| From Pantanelli[1] | 1658 | 2 | 8 | 16 | **29** | 23 | 8 | 8 | | | | | | | | | | | 95 |
| Gordonsville, Va | 1739 | | 10 | 18 | **31** | 24 | 12 | 6 | 3 | 2 | 1 | 4 | 1 | 2 | | | | | 114 |
| Odenton, Md | 1710 | | 1 | 14 | **37** | 25 | 12 | 3 | 1 | | | | | | | | | | 93 |
| Total | | 4 | 30 | 65 | 130 | 94 | 41 | 20 | 7 | 3 | 1 | 4 | 1 | 2 | | | | | 402 |
| Per cent | | 1 | 7.5 | 16.2 | 32.4 | 23.4 | 10.0 | 5.0 | 1.7 | 0.7 | 0.2 | 1.0 | 0.2 | 0.5 | | | | | |

| Specimens. | No. | 1.6 | 1.7 | 1.8 | 1.9 | 2 | 2.1 | 2.2 | 2.3 | 2.4 | 2.5 | 2.6 | 2.7 | 2.8 | 2.9 | 3 | 3.1 | 3.2 | Total. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Number per specimen having the given ratio of lengths to widths. | | | | | | | | | | | | | | | | | |
| Endothia tropicalis: | | | | | | | | | | | | | | | | | | | |
| Ceylon | | | | | 3 | 9 | 22 | **35** | 20 | 9 | 2 | | | | | | | 5 | **43** | **43** | 9 | 100 |
| Do | | | | | 2 | 9 | 17 | **35** | 20 | 13 | 4 | | | | | | | 1 | 28 | 50 | 21 | 100 |
| E. parasitica: | | | | | | | | | | | | | | | | | | | |
| On Castanea sp., Santunying, China | 2151 | | | 8 | 17 | 18 | **26** | 18 | 8 | 3 | 1 | 1 | | | | | | 6 | 27 | **52** | 15 | 100 |
| On Castanea dentata— | | | | | | | | | | | | | | | | | | | |
| From Pantanelli[1] | 1658 | | | 3 | 8 | 15 | 19 | **21** | 15 | 7 | 4 | 3 | | | | | | | 17 | **39** | **39** | 95 |
| Gordonsville, Va | 1739 | | | 7 | **17** | 16 | **32** | 21 | 10 | 7 | 3 | **1** | | | | | | 3 | 43 | **59** | 9 | 114 |
| Odenton, Md | 1710 | | | | 3 | 20 | 26 | **38** | 6 | | | | | | | | | | 13 | **74** | 6 | 93 |
| Total | | | | 18 | 45 | 69 | 103 | 98 | 39 | 17 | 8 | 5 | | | | | | 9 | 100 | 224 | 69 | 402 |
| Per cent | | | | 0.4 | 1.1 | 1.7 | 25.6 | 24.4 | 10.0 | 0.4 | 0.2 | 0.1 | | | | | | 0.2 | 24.9 | 55.8 | 17.2 | |

[1] Specimens of American material sent by Pantanelli, of Rome, Italy.

## PHYSIOLOGY.

### CULTURAL STUDIES.[1]

During the past three years the writers have had under observation more than 4,000 cultures of the several species of Endothia on more than a dozen artificial media, as well as on sterilized twigs of many kinds. Throughout this work the writers have been impressed with the uniformity of the behavior of the organism in culture and the certainty with which the various species could be distinguished on any of the media used.

Cultures of *Endothia parasitica*, for instance, from specimens sent from China or British Columbia were absolutely indistinguishable from cultures made on the same medium from local material. Transfers made from stock cultures which had been kept on artificial media for two years were identical with transfers from freshly collected material. The same remarkable constancy held for the other species. Cultures from material collected in different localities or from different hosts were identical, not only in appearance but, so far as the writers were able to determine, in temperature and moisture relations also. As previously noted, this is in marked contrast to the senior writer's experience with the species of Glomerella and it is believed differs from the experience of many investigators of fungi.

No less striking is the certainty with which the several species may be distinguished on any medium tried. *Endothia parasitica*, *E. tropicalis*, and *E. fluens* and its variety *mississippiensis* are very closely related morphologically. Moreover all except *E. parasitica* have, as near as could be determined, much the same relation to their hosts. Yet each species has distinctly and readily recognized characters on culture media.

It should not be imagined, however, that the differences are recognizable at once as clearly distinctive characters. The differences at first glance might readily be considered fluctuating variations. But the fact that the characters remain constant through hundreds of generations and have never varied toward one another makes them worthy of recognition as specific characters.

In a previous paper (77) the writers described their results with cultures of *Endothia parasitica*, *E. fluens*, *E. fluens mississippiensis*, and *E. gyrosa* on a number of culture media. At that time the work of other investigators was reviewed and the methods of preparing the various culture media and making the cultures described. Since the publication of that paper, however, cultures of two more species, *E. tropicalis* and *E. singularis*, have been secured and about 2,000 additional cultures of the various species made. In addition to the culture media mentioned in the previous paper (77, p. 10), the writers

[1] The cultures described were all grown at ordinary laboratory temperatures in the winter, about 20° to 24° C.

have grown the organisms on sterile twigs of many species and on liquid media.

As stated above, the various species of Endothia are distinguishable on any medium tested. White corn meal in flasks has, however, been most used by the writers in identification work and for keeping stock cultures. All the species grow readily on this medium and may be determined with certainty within 10 days under ordinary conditions of growth. In addition, the medium is cheap, easily prepared, and does not dry out so quickly as agar media in tubes, so cultures may be kept alive much longer without transfers. Almost equally good for purposes of identification are rice and oatmeal in flasks, corn-meal agar, and potato agar.

The distinguishing characteristics of the various species in culture have been described rather fully in the previous publication and may be briefly summarized, as follows:

#### CULTURES ON CORN-MEAL AGAR (UNSLANTED TUBES).

Corn-meal agar proved the best agar medium for the production of pycnospores and showed constant differences in the cultural characters of the various species. The most characteristic differences appeared in cultures from six to eight weeks old on unslanted tubes. (See Pl. XXI, figs. 2 to 7.)

*Endothia gyrosa* at this age showed a rather abundant, felty white mycelium, flecked with capucine buff, but there were no pycnidia. In older cultures small pycnospore threads were sometimes produced. Usually before the cultures were 10 days old the medium was changed to a delicate lavender just below the mycelium, and below this to a light olive green. A few days later the lavender disappeared and the green deepened to olive green.

*Endothia singularis* grew more slowly than any other species. Within three weeks, however, the mycelium covered the entire surface. It was smoother than *E. gyrosa* and nearly white, with raw umber spots where the mycelium touched the glass. The medium was changed to a light hellebore green one-half inch below the top.

*Endothia fluens*, as pointed out in the previous paper, produced an abundant deep-chrome mycelium, with usually one or two rather small pycnidial pustules.

*Endothia fluens mississippiensis* produced a scant surface growth of mycelium, between cadmium yellow and raw sienna in color. The upper one-half centimeter of the agar became reddish orange. The pycnidial pustules were more numerous than those of *E. fluens*, but smaller and more scattered than those of *E. parasitica*.

*Endothia longirostris* at the end of six weeks had a scant, webby, orange, aerial mycelium growing against the glass. Mycelium on the surface of the medium was very scant, orange to cadmium yellow in color, with scattered tiny xanthine orange to orange spore masses. The color of the agar changed to medal bronze just beneath the mycelium, shading into orange citrine below.

*Endothia tropicalis* at the end of six weeks showed a thinly felted mycelium, white to capucine orange, with numerous small, scattered pycnidial pustules. The ring of mycelium against the glass was light orange yellow, as contrasted with white in *E. parasitica*.

*Endothia parasitica* gave a scanty white growth of surface mycelium, with several prominent pycnidial pustules clustered near the center and of a slightly darker shade than the "raw sienna" of Ridgway.[1]

### CULTURES ON POTATO AGAR (SLANTED TUBES).

Potato agar was used by the Andersons (3) to distinguish *Endothia parasitica* from *E. fluens*. The writers have used it extensively and found it a very useful medium for distinguishing the species. As stated in the previous paper (77, p. 11), however, unless this medium was very carefully prepared it varied greatly in acidity and probably in other respects, with resultant variations in the behavior of the organisms. Spore production was not so abundant on this medium as on many others. The preparation of this and other media is described in the paper cited.

*Endothia gyrosa.*—This species developed rather slowly, producing a fairly abundant aerial growth, which was felty rather than fluffy. The color was white, flecked with capucine buff, and no spore masses were produced.

*Endothia singularis.*—This species grew even more slowly than *E. gyrosa*. On cultures made from conidia, growth was hardly perceptible at the end of three days. Mycelial cultures at the end of one week showed less growth than *E. gyrosa*, but did not differ greatly from it in either color or texture. At the end of one month the mycelium was slightly more fluffy and decidedly less in amount than that of *E. gyrosa*. Most of the surface was a very light buff color, with sometimes a few spots of capucine orange to English red.

*Endothia fluens.*—Pycnospore streak cultures of this species varied somewhat as to the amount and time of appearance of color, probably due to the variations in the acidity of the medium referred to above. Many tubes showed an orange color in one week, while others produced no orange whatever. In no case did cultures of *E. fluens* produce the "brassy" metallic surface appearance so characteristic of *E. parasitica*. Pycnidia were few and more scattered than in *E. parasitica* and did not begin to appear until the third or fourth week. A slight amount of warbler-green color sometimes appeared in the medium at this age, but never so conspicuously as in *E. parasitica*.

*Endothia fluens mississippiensis.*—This produced a less fluffy aerial mycelium along the spore streak than *E. parasitica*. After five or six days the fungus showed an orange color by transmitted light, and was indistinguishable in this respect from *E. parasitica*. The character of the surface was somewhat different, however, and by reflected light appeared xanthine orange. When two weeks old this form differed still more markedly from *E. parasitica* in color, being grenadine red by transmitted light and showing no spore masses.

*E. longirostris.*—At the end of one week this produced a white, fluffy growth scattered in small patches over the surface of the medium. This later became rather close in texture, especially near the base of the agar slant. No spores were produced on this medium.

*Endothia tropicalis.*—At the end of one week this showed less growth than *E. fluens*, covering about a third of the surface of the medium, while the other covered nearly the entire surface. The mycelium was closely matted and a very pale buff (paler than any in Ridgway). At the end of one month

[1] In the descriptions of cultures comparisons were necessarily made with cultures in flasks or tubes. This of course made comparison more difficult and somewhat less accurate than if the material had been removed from the container.

*E. tropicalis* covered the entire surface with a thin layer of surface mycelium, considerably darker in color than when one week old.

*Endothia parasitica.*—At the end of three or four days at room temperature this showed a short, fluffy, white, aerial growth along the streak. The surface of the mycelium was orange by transmitted light, while by reflected light it was between raw sienna and antique brown at the sides. Within six days the mycelium, especially at the base of the agar slant, took on a peculiar metallic "brassy" appearance, due apparently in part to the character of the mycelium and in part to the minute water drops scattered over the surface. This portion of the culture was light orange yellow by reflected light and orange by transmitted light. This metallic appearance has been found to be the most constant and reliable distinguishing character of *E. parasitica* on potato agar. In 12 to 14 days small pycnidial pustules appeared in the upper portion of the tubes, and the agar just below the mycelium became warbler-green, changing later to olive green.

CULTURES ON CORN MEAL (IN 100 C. C. ERLENMEYER FLASKS).

*Endothia gyrosa.*—Mycelial cultures one week old showed a growth of rather compact mycelium covering nearly one-half the surface of the medium. The mycelium was ochraceous buff near the point of inoculation, shading into white at the margin. There was no discoloration of the medium and no spore masses were seen.

Cultures of the same kind one month old showed an abundant, rather thick growth, having the surface mostly covered with somewhat irregular tubercular masses, suggesting immature pycnidial stromata similar to those found in *E. radicalis*, but smaller and producing no spores. The surface of the culture was capucine buff, that of the tubercles honey yellow to Isabella. The dark color was apparently due in part to numerous superficial water drops. A portion of the medium was changed to perilla purple.

*Endothia singularis.*—Mycelial cultures one week old covered only one-third of the surface. The growth was mostly white and fluffy, with ochraceous buff near the center.

At the end of one month the growth had entirely covered the surface. The mycelium varied in color from cadmium orange to capucine buff, the color being distributed over the surface in patches. The corn meal was changed to perilla purple near the center. No spores were produced.

*E. singularis* was readily distinguishable from *E. gyrosa*, which it resembled more closely in culture than any of the other species, by the rate of growth and the color and nature of the surface of the mycelium. *E. singularis* grew more slowly than *E. gyrosa*, was rather brighter in color (cadmium orange), and the surface of the mycelium was decidedly more even, lacking the tubercular masses characteristic of *E. gyrosa*.

*Endothia fluens.*—Cultures at the age of one week showed a growth of loose, fluffy mycelium covering one-half of the surface of the medium. The mycelium was deep chrome to light orange yellow at the point of inoculation, passing through perilla purple and light pinkish lilac and fading into white at the margin. Occasionally the medium was changed to perilla purple near the center. No spores were present.

Cultures one month old showed a compact growth, with a nearly smooth surface. The color ranged from light cadmium to empire yellow. The whole mass of the medium was perilla purple. Spore masses were rarely present at this stage, but shortly afterwards a few large erumpent stromata were formed, which extruded spores in thick masses.

*Endothia fluens mississippiensis.*—Cultures one week old showed an orange-chrome growth a little more than half covering the surface of the medium. The superficial growth was very similar to that of *E. parasitica.* There was no discoloration of the medium and no spore masses were found.

The same organism one month old produced a growth with a compact, rather uniform surface, the superficial portion having a coarse, matted, webby appearance, which was most noticeable about the margin. The color of the mycelium was cadmium orange to xanthine orange, while that of the medium was unchanged. Spore masses were much more numerous than in *E. fluens,* but smaller and less numerous though very similar to those of *E. parasitica.*

*E. longirostris.*—Cultures one week old covered about one-third of the surface of the medium. The mycelium was short, fluffy, white, with only a tiny spot of cadmium orange near the point of inoculation. At the end of six weeks the entire surface was covered with a compact growth rather uniform in texture, cadmium orange to xanthine orange in color. The surface was irregularly ridged, giving it a wrinkled appearance, with tiny mars orange spore masses irregularly scattered over the surface. This species closely resembles *E. fluens mississippiensis* on this medium, being distinguished from that variety by the smaller and much less numerous spore masses. The medium is changed to amber brown just below the mycelium, shading into mars yellow in the lower portions.

*Endothia tropicalis.*—At the end of one week this showed less growth than either *E. parasitica* or *E. fluens,* covering about a third of the surface. The mycelium was matted close to the surface and was a very pale buff (paler than any of the buffs shown in Ridgway). No pycnidia were present.

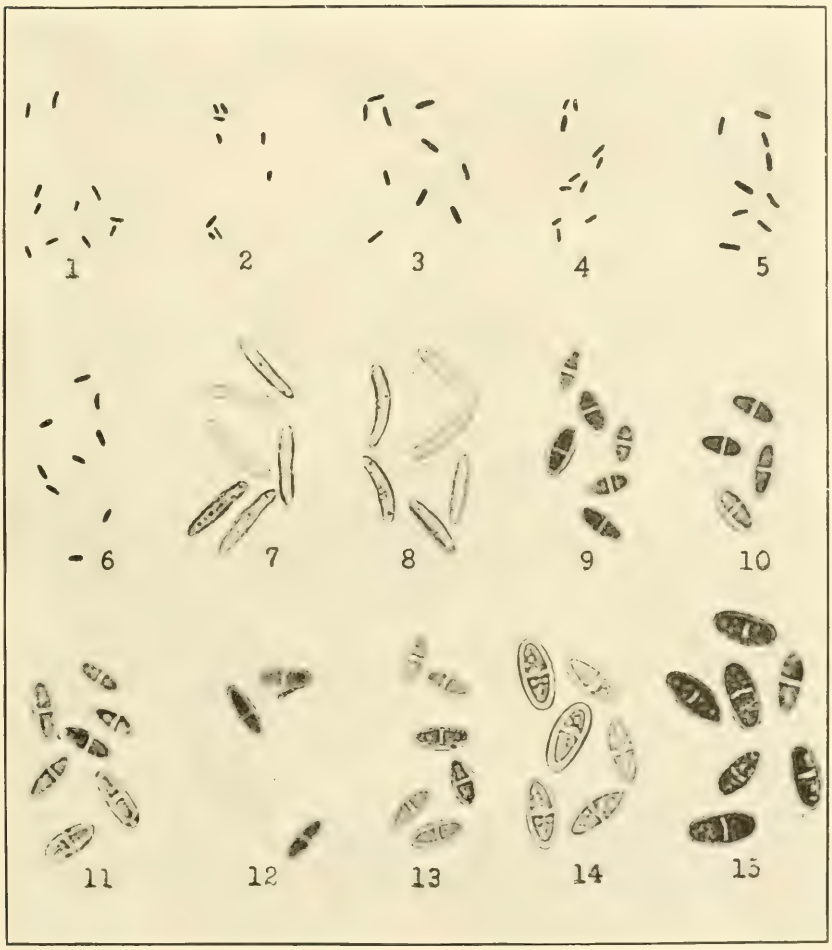
At the end of one month's growth the surface was entirely covered with a closely felted mycelium and small, numerous, thickly scattered spore masses, more closely resembling those of *Endothia parasitica* than any other species. The mycelium was orange buff to apricot orange, and orange chrome against the glass. The color of the medium was unchanged.

*Endothia parasitica.*—In cultures one week old the growth on corn meal covered about one-half of the surface of the medium. The outer margin was pure white, the remainder buff yellow below, with a superficial white growth above. A few small pustules with spore masses occurred near the point of inoculation. The medium was uncolored.

Cultures one month old showed a compact growth, nearly smooth on the surface. The superficial mycelium was pale orange yellow. The pale yellow-ocher spore masses were minute, very numerous, and nearly covered the surface. The medium was slightly greenish about the sides of the flask just beneath the mycelium.

### DISTINGUISHING CHARACTERS OF THE VARIOUS SPECIES ON CORN MEAL IN FLASKS.

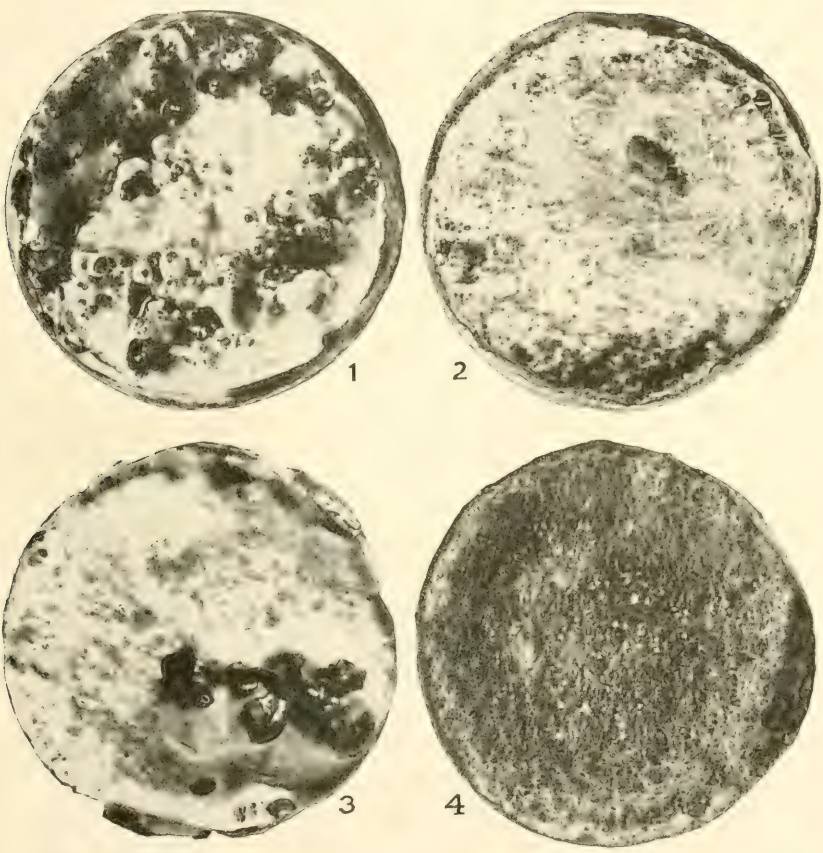
The color reactions of the various species on corn meal are very striking. *Endothia fluens* (Pl. XXI, fig. 1*b*), as noted above, changes the whole mass of the medium to perilla purple in less than a month. *E. gyrosa* and *E. singularis* also produce this color change, but somewhat more slowly. *E. fluens mississippiensis*, *E. tropicalis*, and *E. parasitica*, on the other hand, in hundreds of cultures have wholly failed to produce any purple color. This furnishes an easy and reliable method of distinguishing *E. parasitica* from *E. fluens*




PHOTOMICROGRAPHS OF PYCNOSPORES AND ASCOSPORES OF ENDOTHIA.

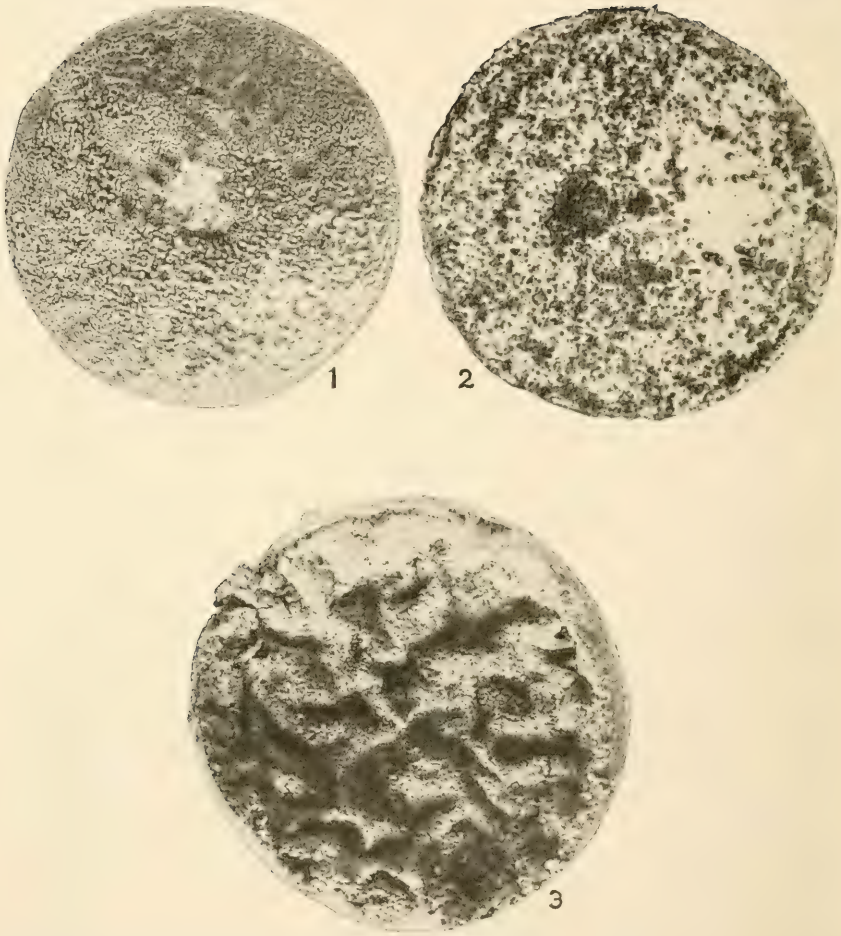
FIGS. 1 TO 6.—PYCNOSPORES: 1, ENDOTHIA GYROSA; 2, E. SINGULARIS; 3, E. FLUENS; 4, E. LONGIROSTRIS; 5, E. PARASITICA (AMERICAN); 6, E. PARASITICA (CHINESE). FIGS. 7 TO 15.—ASCOSPORES: 7, E. GYROSA; 8, E. SINGULARIS; 9, SPHAERIA RADICALIS, FROM SCHWEINITZ'S SPECIMEN IN FRIES'S HERBARIUM; 10, ENDOTHIA PSEUDORADICALIS; 11, E. FLUENS; 12, E. FLUENS MISSISSIPPIENSIS; 13, E. LONGIROSTRIS; 14, E. TROPICALIS; 15, E. PARASITICA.

ENDOTHIA PARASITICA ON PLATE CULTURES OF CORN-MEAL AGAR 4 WEEKS OLD. THE UPPER PLATE WAS KEPT IN TOTAL DARKNESS; THE LOWER PLATE IN THE DIRECT LIGHT OF A NORTH WINDOW.




ENDOTHIA SPECIES ON WHITE CORN MEAL (10 GRAMS OF CORN MEAL TO 20 C. C. OF WATER). CULTURES 2 MONTHS OLD.

FIG. 1.—ENDOTHIA GYROSA; FIG. 2.—E. SINGULARIS; FIG 3.—E. FLUENS; FIG. 4.—E. FLUENS MISSISSIPPIENSIS.




ENDOTHIA SPECIES ON WHITE CORN MEAL (10 GRAMS OF CORN MEAL TO 20 C. C. OF WATER). CULTURES 2 MONTHS OLD.

FIG. 1.—ENDOTHIA TROPICALIS; FIG. 2.—E. PARASITICA; FIG. 3.—E. LONGIROSTRIS.

CULTURES OF ENDOTHIA SPECIES

FIG. 1.—*a*, Corn meal after 1 month's growth of *Endothia parasitica*; *b*, corn meal after 1 month's growth of *E. fluens*.

Typical cultures on upright tubes of corn-meal agar 6 weeks old. FIG. 2.—*E. gyrosa*. FIG. 3.—*E. singularis*. FIG. 4.—*E. fluens*. FIG. 5.—*E. fluens mississippiensis*. FIG. 6—*E. tropicalis*. FIG. 7.—*E. parasitica*.

(Pl. XXI, fig. 1) in field work when fructifications of the species are wanting or doubtful.

Aside from the differences in color, the most conspicuous and important characteristic of these fungi in corn-meal cultures is found in the fructification. Clinton (18, pl. 26) has already mentioned and illustrated similar differences in cultures of these organisms on agar in Petri dishes. In *Endothia parasitica* the pycnidia and spore masses are small, numerous, thickly scattered, and embedded in the mycelium. *E. fluens*, on the other hand, forms few, large, erumpent stromata, with spores extruding in thick, elongated masses. *E. tropicalis* closely resembles *E. parasitica* in number, size, and arrangement of pycnidia and spore masses, but differs in color of mycelium. *E. fluens mississippiensis* appears somewhat intermediate between *E. parasitica* and *E. fluens* in regard to the character and abundance of the pycnidia and in color of the growth. These peculiarities have been very uniform and constant in all the cultures on this medium and if they could be coordinated with regular morphological differences in nature would justify the separation of this form as a species. (See Pls. XIX and XX.)

### CULTURES ON LIQUID MEDIA (IN 100 C. C. FLASKS).

Some difficulty was experienced at first in growing the species of Endothia satisfactorily on a liquid medium. Abundant growth was obtained on a medium suggested by Dr. Mel. T. Cook. This is a modification of the liquid medium No. II as given by him (19).

Cook's liquid medium, No. II, is prepared as follows:

Into 500 c. c. of distilled water put 15 grams of glucose and 20 grams of peptone steamed at 100° C. for three-fourths hour; into another 500 c. c. of distilled water put 0.25 gram of dipotassium phosphate and 0.25 gram of magnesium sulphate, steamed for 20 minutes; filter both 500 c. c. into same receptacle, steam 10 minutes, put into flasks, about 30 c. c. in each flask, and autoclave.

All species grew readily on this medium, *Endothia parasitica* even producing pycnospores. At the end of one month's growth the several species were readily distinglished on this medium and may be briefly described as follows:

*Endothia gyrosa.*—Growth scanty; did not form a continuous mat, but remained in small bunches, giving an almost flocculent appearance. The mycelium appeared white when removed from the culture solution, but the solution itself was honey yellow.

*Endothia singularis.*—Growth even less abundant than *E. gyrosa;* formed small brown knots against the glass. Mycelium buff, and the medium was changed to honey yellow.

*Endothia fluens.*—Growth somewhat more abundant and less closely matted than *E. parasitica*, entirely submerged; mycelium white; liquid unchanged in color.

*Endothia fluens mississippiensis.*—Growth slightly less abundant than in *E. parasitica;* submerged except at the very edges; much lighter in color, being reddish brown.

*Endothia tropicalis.*—This differed markedly from either *E. parasitica* or *E. fluens.* The mycelium formed a thin felt over the surface, white to salmon orange in color, with no change in the medium.

*Endothia parasitica.*—Mycelial growth very abundant, closely matted, chiefly submerged, but slightly arborescent in one or two small areas, which remained above the surface. Color, dark greenish brown.

### CULTURES ON STERILIZED TWIGS (IN TUBES).

Early in this work it was noted that all the species of Endothia grew readily on sterilized chestnut twigs in test tubes. Later, tests were made with twigs from a number of common, woody plants. Twigs of *Acer saccharum*, *Alnus rugosa*, *Betula papyrifera* and *B. lenta*, *Carpinus caroliniana*, *Cornus florida*, *Fagus grandifolia*, *Fraxinus americana*, *Ostrya virginiana*, *Populus grandidentata*, *Prunus serotina*, *Rhus glabra*, *Tilia americana*, and *Tsuga canadensis* were collected in New York State early in June, placed in test tubes with a few cubic centimeters of distilled water and sterilized in an autoclave. All the species of Endothia were tested, and all grew on every species of twig except Tsuga. The difficulty of completely describing this series may readily be seen from the fact that each species of Endothia had a different appearance on every kind of wood.

In general it may be stated that *Endothia gyrosa* and *E. singularis* grew more slowly than the other species and produced no spores, while all the other species produced spores on most hosts. The mycelium of *E. parasitica* was usually white, especially on the bark. *E. gyrosa* and *E. singularis* produced various shades of buff, while *E. fluens*, *E. fluens mississippiensis*, and *E. tropicalis* developed a much more brightly colored mycelium, usually showing yellow or orange shades.

### MOISTURE RELATIONS.

In an earlier paper (77, p. 7) the writers reported tests with *Endothia fluens* and *E. parasitica* on media containing various percentages of water. It was observed that pycnospore production began earliest and was most abundant on the media containing the least moisture.

Aside from this the writers have thus far been unable to make definite tests as to the moisture relations of these fungi. However, incidental observations in connection with the light tests (p. 43) and temperature tests (p. 45), as well as results of field experiments, particularly those at Woodstock, N. Y., make it apparent that the amount of available moisture is a very important factor in the fructification of the fungus.

### LIGHT RELATIONS.

The relation of light to pycnospore production in *Endothia parasitica* was first discussed by Anderson (1, p. 20). He says—

> When plate cultures are grown in total darkness on chestnut-bark agar, no pycnidia are developed, while on plates made at the same time and grown in the light, the usual rings of pycnidia appear (fig. 57). Experiments were also tried in which the plate was left in darkness until about half covered with mycelium and then brought into the light. Circles of pycnidia were developed, beginning with the ring which marked the outermost limit of the colony when removed from the dark chamber. The concentric rings which always appear on agar cultures are due to the alternation of night and day.

Later, in a bulletin by Anderson and Rankin (6, p. 592), the same results are attributed to D. C. Babcock.

Up to the time the above-mentioned work was published the writers had grown about 3,000 cultures of the several species of Endothia on various media in flasks and tubes. Practically all of these cultures had been kept in dark cases and *Endothia parasitica* had produced pycnidia abundantly on most of the media used. It seemed desirable, therefore, to determine whether wholly different light relations existed when the fungus was grown on plates. The following series of tests was accordingly made, using *E. parasitica* only.

### LIGHT TESTS OF CULTURES ON PLATES.

In experimenting with plate cultures in order to check up the results reported by Anderson and Rankin (6, p. 592) it was noted that there was great variation in the rate at which the cultures dried out. There was considerable variation in this respect in different plates kept side by side, apparently due to differences in the Petri dishes, and a marked difference between cultures kept in light and those kept in darkness. Since a causal relation between lack of moisture and abundant spore production had already been shown, it seemed probable that this might influence the results of the light tests in plate cultures. In fact, in a few cases the cultures kept in the light did produce spores earlier than those kept in darkness. Accordingly, in order to eliminate at least in part this fact which seemed to obscure the possible effect of light, a method was sought of equalizing the loss of moisture. In the following series half the plates were placed under a plain bell jar and the other half under a bell jar of equal size but darkened by being covered inside and out with heavy black paper, such as is used to wrap photographic plates. The two bell jars were then set side by side in front of a north window. By this means the conditions were made much more uniform as to temperature and moisture. There was still a slight difference in the rate of drying and undoubtedly at times a difference

in the temperature of the light and dark plates, but probably not sufficient to interfere seriously with the experiments.

*Series 1. On corn-meal agar plates under bell jars.*—In nine days there was no distinguishable difference between the plates in light and darkness, a few spore masses occurring near the middle of each.

In 18 days most of the light plates showed a central ring of spore masses and a zone of scattered spore masses near the edge. The dark plates showed a few small spore masses near the center, and scattered about the outer portion were the small masses of mycelium which usually constitute the early stages of pycnidial formation.

In 30 days the number of spore masses had increased somewhat in both sets of plates, but more in the darkened plates, so that the number of spore masses was about equal in all the plates. The two sets of plates were fairly uniform as to the arrangement of the spore masses. Plate XVIII shows a typical example.

*Series 2. On chestnut-twig agar plates under bell jars.*—After nine days the cultures in light and darkness were alike. No spores had yet appeared in either set.

In 30 days there were a few spore masses on nearly all of the plates, there being no difference between those in light and those in darkness either in number or distribution.

*Series 3. On corn-meal agar and chestnut-twig agar under bell jars.*—In this test the plates were piled alternately, first a corn-meal and then a chestnut-twig agar plate, so that the two media would be under conditions as nearly identical as possible. The plates were inoculated as before and left untouched for 18 days and after that were examined daily. After 18 days all the corn-meal plates showed spore masses in practically equal numbers, while the chestnut-twig agar plates showed no spore masses whatever. There was no apparent difference in the growth on either medium between the plates in light and those in darkness.

At the end of 25 days the cultures on chestnut-twig agar plates showed numerous small masses of mycelium, indicating the formation of pycnidia. No difference was perceptible between the dark and light plates.

In 28 days, from 100 to 150 of these pycnidia in each plate were extruding spore masses. The light plates showed in general a larger mass of spores than the dark plates, but this was not marked, certainly no greater than was accounted for by the unavoidable difference in radiation and the consequent difference in moisture. This difference in the moisture of the medium was clearly shown each morning by the greater amount of moisture condensed on the covers of the darkened Petri dishes.

At this time (after 28 days) four corn-meal agar plates which had been wrapped in four layers of heavy black photographic paper and

placed on a window sill were opened and examined. In spite of the cold weather prevailing during this test and the consequent low temperature of the room at night, these plates contained an average of nearly 200 well-developed spore masses.

At the end of 35 days the chestnut-twig agar plates which had been kept in the light showed an average of 160 spore masses, while those kept in darkness showed an average of 130 spore pustules, a comparatively small difference in favor of the light plates. There was, however, a wide difference between the various plates in each series, and it was impossible in most cases to distinguish cultures grown in the light from those grown in darkness either by the number, size, or arrangement of the pycnidia and spore masses.

From these experiments it is evident that pycnidia are produced abundantly in total darkness on chestnut-twig agar as well as on other favorable media. There is no perceptible difference in the amount of spore production or in the arrangement of pycnidia between cultures kept in total darkness and those kept in the light during the day if the temperature and evaporation remain the same in both. Continued observation of numerous cultures grown both in daylight and in darkness has convinced the writers that light has no perceptible effect on mycelial growth either in amount, nature, or color production. It seems evident, therefore, that light is a negligible factor in the growth and fructification of these fungi.

### TEMPERATURE RELATIONS.

In an earlier paper (77, p. 9) the writers published the results of three series of tests made to determine the temperature relations of three species of Endothia. Since the publication of that paper cultures of other species and additional material of some of the species from widely separated localities have been secured. Four series of temperature tests including this new material were made on solid media.

#### TESTS ON SOLID MEDIA.

In these tests cultures of *Endothia gyrosa*, *E. singularis*, *E. fluens*, *E. fluens mississippiensis*, and *E. parasitica* were tried on corn-meal agar in slanted tubes, oatmeal in flasks, and potato agar in slanted tubes. The cultures tested were from specimens chosen from the extremes of the known ranges of the fungi and from their different hosts. No difference could be detected in the various cultures of the same species, even in those from widely separated localities and from different hosts. Cultures appeared to have the same temperature relations whether made from spores or mycelium. The results may be briefly summarized as follows:

At 41° and 39° C. there was no growth in any species. Cultures removed from the incubator at the end of 11 days and kept at room temperature showed no growth.

At 35° C., *Endothia gyrosa*, *E. singularis*, and *E. parasitica* showed a slight development within 2 days, but at the end of 11 days it was still slight and abnormal in appearance. *E. fluens* and *E. fluens mississippiensis* showed no growth at this temperature.

At 31° C., *Endothia gyrosa*, *E. singularis*, and *E. parasitica* appeared about the same as at room temperature for the first four days. At the end of six days these species showed somewhat less growth than at room temperature, while at the end of two weeks the growth was less in extent and markedly less freshly colored than that at room temperature. *E. fluens* and *E. fluens mississippiensis* showed somewhat less growth than at room temperature even in 4 days, and markedly less at the end of 2 weeks.

At room temperature (which at this time varied from 20° to 24° C.) the growth was much as described in the previous paper. Within 11 days growth was practically complete and in 14 days there was abundant spore production in *Endothia parasitica*.

At 18° and 16° C., all species showed considerably less growth than at room temperature, but there seems to be little difference in the comparative growth of the various species at these temperatures. At 13° the growth was decidedly less than at 16° C. but was fairly normal in appearance in all the species except that *Endothia fluens mississippiensis* failed to produce the characteristic color at this temperature.

At 9° C. there was a very slight growth in all species.

At 7°, 5°, and 2° C. there was no growth whatever. Cultures removed to room temperature at the end of 11 days developed normally and at about the same rate as in newly made cultures.

These additional tests seemed to confirm the results already published (77, p. 27); that is, growth was best in all species at ordinary room temperature, about 20° to 24° C. The minimum temperature for all was about 9°, and all failed to grow at 7° C. The maximum temperature for *Endothia gyrosa*, *E. singularis*, and *E. parasitica* appeared to be about 35°, while the maximum for *E. fluens* and its variety *E. fluens mississippiensis* was apparently about 32° C. At all the temperatures tried *E. singularis* grew much more slowly than any of the other species.

It was noted that cultures kept at 7°, 5°, and 2° C. showed no growth, but when removed to room temperature developed normally, while cultures kept at 41° and 39° C. failed to grow when removed to room temperature. This seemed to indicate that the fungi are more susceptible to heat than to cold, and such is perhaps the case. There was, however, the additional factor of moisture involved, for while the agar of the cultures kept at 7° and lower was in apparently the same condition at the end of 11 days as when first inoculated, the agar of the cultures kept at 41° to 39° C. was considerably dried. This raised the question as to whether the drying out of the agar had not affected the growth of the fungi in those cultures kept above room temperature as much as the higher temperatures themselves.

The same idea was suggested by the fact that several of the species grew for a few days at 31° C. as well as they did at room temperature, and then fell behind. It seemed possible that this falling off in the

rate of growth might be due, at least partly, to more rapid drying of the agar at 31° C., or possibly to the more rapid development of some toxin, as was suggested by Balls (7) to explain a similar observation on the "soreshin" fungus. These observations threw doubt upon the accuracy of the writers' previous conclusions, and made it seem possible that the optimum temperature of the species of Endothia might be well above room temperature. This could only be determined accurately by some method which would control temperature without altering the supply of moisture. Some months after the above tests were concluded it was discovered that the various species of Endothia would grow readily on several liquid media. Consequently, several series of tests on liquid media were run parallel to those described above, except that the tests were continued for only four days. Experiment showed that at the higher temperatures the medium became considerably reduced by evaporation if left for a longer period.

### TESTS ON LIQUID MEDIA.

In the series of tests on liquid media, all the species of which cultures had been obtained were grown on Cook's medium (see p. 41) both in tubes and in flasks, using ten tubes and six flasks at each temperature. The cultures of *Endothia gyrosa* and *E. singularis* were made with bits of mycelium from pure cultures. The other species were grown from conidia and the cultures were kept for two days at room temperature, in order to allow the conidia to germinate before being placed in the temperatures to be tested.

The following temperatures were used for making the tests: 40°, 37.5°, 35°, 29°, and 27°, and room temperature which was fairly constant at about 22°, 17°, 12°, 9°, 7°, 3°, and 2° C. There was some variation in the temperature of the incubators and refrigerators used, but in most cases they did not vary more than 1 degree above or below the temperature indicated. At 40° there were occasional traces of growth, especially in *Endothia parasitica*, but this may have occurred when the incubator dropped to 39° C. There is no regular and continued growth at this temperature.

At 37.5° C. there was perceptible growth in all the species. This is in striking contrast to the results on solid media, as no species grew at a temperature above 35° C. on solid media.

At 35° C. *Endothia parasitica* showed practically the same amount of growth as at 27° and 29° C. for the first three days, but fell behind after that. *E. fluens* showed less growth at 35° than at the lower temperatures. These two species were the only ones tested at 35° C.

At 27° and 29° C. growth was markedly more abundant than at 37.5°, and in most of the species was more abundant than at room temperature. In *Endothia gyrosa* and *E. fluens mississippiensis* the

growth at 27° C. was apparently equal to that at room temperature. At 22° C. (room temperature) all species developed much more rapidly than at the lower temperatures. At 17°, 12°, and 9° C. there was progressively less and less growth. At 7° C. and lower there was no growth whatever.

While these tests are not wholly satisfactory and must be regarded only as approximations, they are of some interest. Below 7° C. there is no growth in any species.

It is evident that there is a considerable range of temperature, from below 20° to well above 30° C., within which the species of Endothia grow readily. Within this range there may be a definite optimum for each species, but this has not yet been determined. For *Endothia parasitica* the optimum appears to be at 27° C. or above, and the same may be true of the other species.

At 40° C. or above no growth occurs. There is considerable evidence, however, that *Endothia fluens* is less resistant to the higher temperatures than either *E. parasitica* or *E. gyrosa*. After several of the tests the flasks were kept at room temperature for some days. It was found that all developed normally except those which had been kept at 40° and 37.5° C. These developed more slowly than those which had been kept at lower temperatures. It was particularly noticeable also that *E. parasitica* and *E. gyrosa* developed practically as well after being kept at 40° as at 37.5° C., while cultures of *E. fluens* which had been in 37.5° developed fairly well; but if kept at 40° for three days they entirely failed to develop.

## DISTRIBUTION OF THE SPECIES OF ENDOTHIA.

During the past two years the writers have studied over 600 specimens of Endothia from various parts of the world. The greater number of these specimens have naturally come from the United States. The maps (figs. 1–4) show the known ranges of the various species in this country. Each dot on a map represents a locality from which the species has been collected. Frequently, of course, many specimens have come from a single locality; hence the number of dots by no means represents the number of collections.

In the case of *Endothia parasitica*, the dark portion represents the area over which the blight is practically continuous; that is, practically all the stands of chestnut are either diseased or dead. The dots represent known isolated infections and the solid line marks the botanical limit of the chestnut.

*Endothia gyrosa* is known only from the United States, but has a range in this country wider than that of any other species. As shown in figure 1, it has been found as far north as central Michigan, east to Connecticut, on the Pacific coast near San Francisco, and on

the Gulf of Mexico. There is, however, a very great difference in the abundance of this species at different points. In the southeastern United States—that is, the region south of central Indiana and southern Virginia and east of central Arkansas and Louisiana—this species occurs in great abundance wherever its hosts are found. Broken branch stubs and exposed roots of Liquidambar, Fagus, and Quercus are covered with fructifications of this fungus. This is especially true of roots exposed by erosion or excavation which have suffered mechanical injury through the tramping of men or cattle. Farther north in Maryland, New Jersey, and Connecticut only an occasional specimen is found. Three days' search in southern Con-

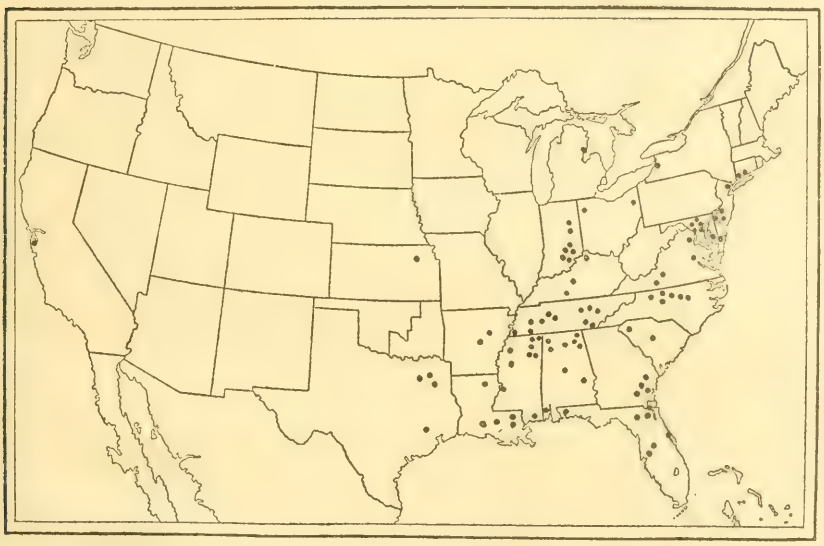


FIG. 1.—Outline map of the United States, showing the distribution of *Endothia gyrosa*.

necticut, for example, yielded only two specimens, both on Liquidambar, showing pycnidia only.

*Endothia singularis* is known at present only on oaks in the dry foothill regions of Colorado and New Mexico. Bethel, in a letter, states that it is very abundant in certain localities in Colorado.

*Endothia fluens* has long been known to occur in both Europe and America. Recently, through the kindness of Dr. Y. Kozai, director of the Central Agricultural Experiment Station, Nishigahara, Tokio, Japan, the writers received four specimens of fungi on chestnut. One of these, collected by S. Tsuruta on October 14, 1914, in the Province of Totomi, was the pycnidial stage of an Endothia, which when cultured proved to be *E. fluens*. Ascospore material of this species has since been collected by Meyer at Nikko, Japan, on the bark of *Pasania* sp.

*Endothia fluens*, while common to Europe, Asia, and America, has a much more limited range in the United States than *E. gyrosa*. It is fairly common on Castanea and Quercus from southern Pennsylvania and Ohio to northern Mississippi and Alabama. In southeastern Pennsylvania it has been found so far only on roots of Quercus, and in northern Mississippi and Alabama only on *Castanea dentata*.

*Endothia fluens mississippiensis* was first sent to the writers from Corinth, Miss., by Mr. T. E. Snyder, of the Bureau of Entomology, and has since been collected in only four other localities, three near the northeastern corner of Mississippi and one in central Kentucky.

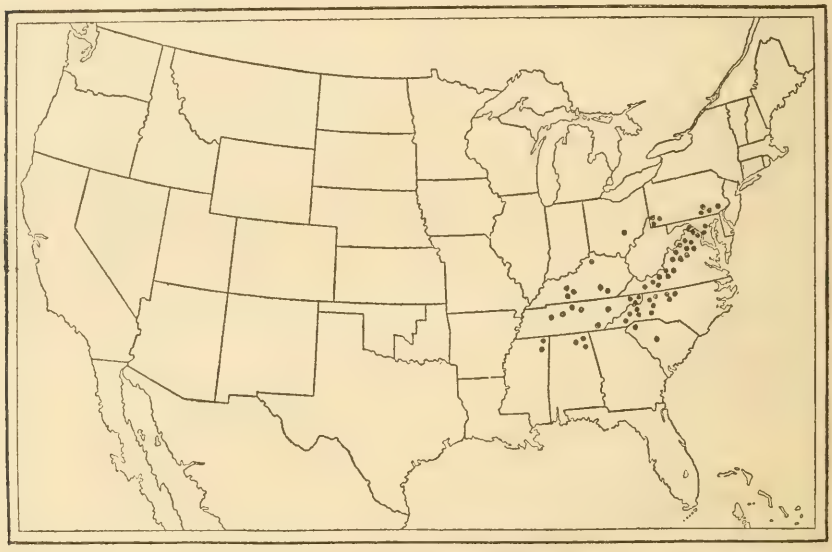


FIG. 2.—Outline map of the United States, showing the distribution of *Endothia fluens*.

As both *Endothia gyrosa* and *E. fluens* were collected in this country nearly a century ago by Schweinitz, it seems altogether probable that they are indigenous species which may have already reached the limits of their natural ranges in this country.

While the maps (figs. 1–4) do not give by any means every locality where Endothia is to be found and specimens are likely to be collected at many points outside the present known range, the writers feel justified in assuming that these maps represent the limits of the territory where *Endothia gyrosa* and *E. fluens* may commonly be found. This is especially true in the eastern portion, where the field has been rather thoroughly worked. It is unlikely, for instance, that *E. fluens* occurs abundantly in southern Alabama and Georgia, where *E. gyrosa* was found so commonly. Southeastern Pennsylvania must be somewhere near the northern limit for *E. fluens*, for the writers' four collections in that region are the result of six days'

search. At the northeastern limit of *E. gyrosa*, Clinton (15, p. 79) found only a single specimen after two years' search, and the writers have looked for it in all the other New England States without finding a single specimen. The report of *E. gyrosa* from Massachusetts by Hitchcock (42, p. 63) has already been shown to be a probable error in identification.

## FACTORS INFLUENCING DISTRIBUTION.

### HOST RELATIONS.

Just what determines the present ranges of the species can not, of course, be positively decided, but some relation to certain external factors may be traced. Neither species has the same distribution as

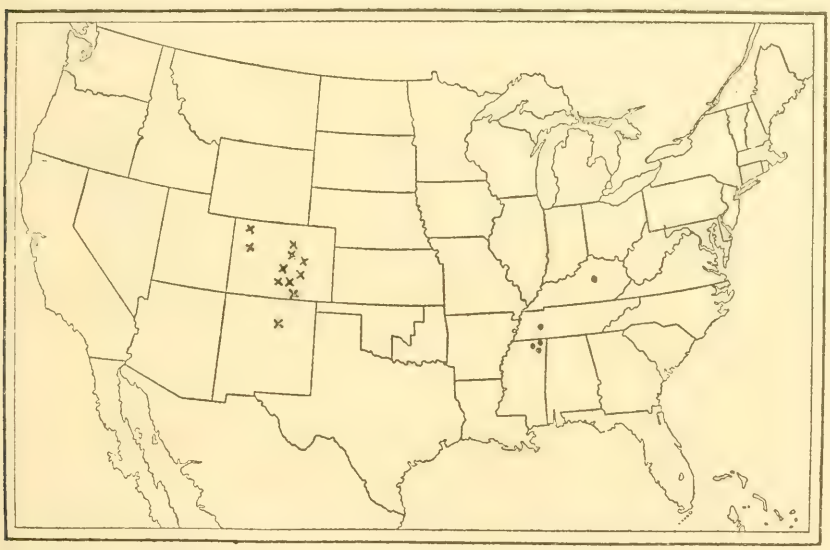


FIG. 3.—Outline map of the United States, showing the distribution of *Endothia fluens mississippiensis* and *E. singularis*. The dots indicate collections of *E. fluens mississippiensis* and crosses indicate collections of *E. singularis*.

its hosts. Quercus and Fagus are both abundant farther north than *Endothia gyrosa* has yet been found, while Quercus is abundant north and south of the known range of *E. fluens*. It may be worthy of note, however, that *E. gyrosa* extends north as far as Liquidambar is found. Perhaps more significant is the relation of the range of *E. fluens* to that of the chestnut. As will be seen from a comparison of the maps (figs. 2–4), *E. fluens* is not found abundantly at any point outside the natural range of the chestnut. Especially interesting is the fact that the southeastern limits of *E. fluens* and *Castanea dentata* are practically coincident, for in this region *Endothia fluens* was found only on Castanea, never on Quercus. This suggests the possibility that Castánea may be the original and favorite host of *Endothia fluens*.

SOIL CONDITIONS.

Greater opportunity for infection seems to be an important factor in the greater abundance of Endothia in the South. By far the most favorable places of infection, especially for *Endothia gyrosa*, are bruised or broken but still living roots. Soil, cultural, and climatic conditions combine to make these many times more abundant in the Southern States than elsewhere. The more sandy and easily eroded soil, usually without turf, subject throughout the winter to the action of wind and rain, leaves innumerable oak roots exposed, which are readily injured by vehicles and the tramping of horses and cattle, leaving wounds suitable for the entrance of Endothia. In the

FIG. 4.—Map of the United States, showing the distribution of *Endothia parasitica* in December, 1915. The solid portion shows the area in which *E. parasitica* is generally present. The dots indicate scattered infections. The heavy line shows the limits of the range of *Castanea dentata*.

North, the more rocky soil, frequently covered with sod, protected through much of the winter by snow, makes exposed roots much less common, and the roots so exposed are rather less subject to mechanical injury. In the writer's experience the most favorable localities for collecting *E. gyrosa* are the unfenced public squares of Southern towns, where partial grading, erosion, and constant traffic have left hundreds of oak roots exposed, and the pastures of southwestern Indiana, where the roots of Fagus are often found injured by cattle.

COMPETITION AMONG FUNGI.

The writers' extensive field studies and observations have convinced them that competition among fungi must be considered as a

factor in determining their distribution. As already stated, while *Endothia fluens* occurs on Quercus, it has been found toward the southwestern limit of its range (northern Mississippi and Alabama) only on Castanea, and in Tennessee the writers have sixteen collections of this species on Castanea and only three on Quercus. In this same region, *E. gyrosa* is everywhere abundant on Quercus. In numerous inoculations with *E. gyrosa* and *E. fluens* on oak it has been found that *E. gyrosa* is more generally successful than *E. fluens*. Moreover, *E. gyrosa* occurs abundantly on Liquidambar and Fagus in this region, thus providing more numerous sources of infection for this species than for *E. fluens*. It seems highly probable, therefore, that *E. gyrosa*, with its greater affinity for oak and greater opportunity for infection, may occupy the available oak roots to the exclusion of *E. fluens*, even though climatic conditions are favorable for the growth of the latter species. Castanea rarely serves as a host for *E. gyrosa;* consequently, on this host *E. fluens* meets with little competition and is very abundant.

In the northeastern limit of its range, *Endothia fluens* has been found only on oak roots. Whether it grows naturally on chestnut in this region can not well be determined, since practically all the chestnut trees here are dead or badly diseased with *E. parasitica*. *E. gyrosa* is rare in this region, but *E. fluens* here evidently comes into competition with *Valsa frustum-coni* (Schw.) Curtis, which is common on exposed roots of various species of Quercus.

### CLIMATE.

Since none of the species of Endothia in America extends to the limits of its host species, climate probably has an important part in determining their present ranges.

In this connection it is of interest to compare several life zone and climatic maps which have been published with the range maps of the various species of Endothia. The map entitled "Life zones of the United States," by C. Hart Merriam (50, pl. 14), is based largely on a study of animal life. Merriam deduces from his studies the conclusion that the northward distribution of animals and plants is determined by the total quantity of heat and their southern distribution by the mean temperature of the hottest part of the year. The life zones which he outlines show, however, a striking relation with the known ranges of Endothia in America. With the exception of a single locality for *Endothia gyrosa* in Michigan, all the known localities for *E. gyrosa* and *E. fluens* fall within the Upper Austral and Lower Austral zones. All the known localities for *E. fluens* and all the region where *E. gyrosa* has been found abundantly fall within the humid divisions of these zones. The northeastern limits of the Upper Austral coincide very closely with those of *E. gyrosa;* its

limits in Pennsylvania include the northern localities known for *E. fluens*, while the southern limits of this zone coincide closely with the southern limit of *E. fluens*.

The Livingstons (47) have published maps based on temperature summations and temperature efficiencies, as well as maps in which isoclimatic lines of temperature are combined with precipitation indices and evaporation indices for the mean frostless season.

While no very definite relations between these maps and the ranges of Endothia can be traced it is noteworthy that the localities where *Endothia gyrosa* is known to be abundant are all south of or near the 600 line of temperature efficiency, and only one collection of *E. gyrosa* has been made north of the 400 line. *E. singularis*, on the other hand, has thus far been found only north of the 400 line.

Zon's map (86) of vegetal regions of the United States is based on periods of growth and rest. The regions where *Endothia gyrosa* and *E. fluens* are abundant are all south of the line which marks the northern limit of seven months' vegetation. In fact this coincides very closely with the northern limit of *E. fluens*, and no specimen of *E. gyrosa* showing ascospores has been found farther north.

The relations pointed out above strongly suggest the possibility of some causal connection between climatic conditions and the present ranges of Endothia species, but just what factors may limit the spread of the species is not yet determined. The temperature tests recorded on pages 45 to 48 throw little light on this problem, for the maximum and minimum temperatures are about the same in the various species. *Endothia fluens* seems to be less resistant to the effects of high temperature (40° C.), but it is difficult to see that this fact alone has any direct bearing on the question of distribution.

## DISCOVERY OF ENDOTHIA PARASITICA IN CHINA.

For eight years after its discovery in the New York Zoological Park in the summer of 1904, *Endothia parasitica* was known only from eastern North America. During this time two quite different opinions as to the origin of the fungus were advanced. Some investigators maintained that *E. parasitica* was an indigenous fungus (15); others that it had been imported from some foreign country, probably oriental (51, 52.) In the fall of 1912, however, pycnospore material was sent from Agassiz, B. C., by H. F. Güssow, Dominion Botanist of Canada. Cultures made from this material were identical with *E. parasitica*, and a series of inoculations on *Castanea dentata* produced typical cankers. Later, a large quantity of material collected at Agassiz by Dr. James R. Weir was received, which included a few ascospores. These proved to be typical *E. parasitica*.

A brief description of the identification of other specimens of *E. parasitica* from Agassiz is given by Faull and Graham (29). These writers report that in the material sent them in the summer of 1913 there were no perithecia, but that the pycospores were typical *E. parasitica* and the characteristic mycelial fans were present in the bark. Cultures of the fungus proved it to be identical with *E. parasitica*. They also state (p. 203) that the chestnuts grown at Agassiz "are of oriental, European, and American origin. The stock was purchased from nursery firms located in New Jersey, Ohio, and California. One of these at least 'was a heavy importer of oriental trees and shrubs'." They suggest that it "is significant that a connection with the Orient exists."

In support of this view, the statement of Mr. Sharpe, who had charge of planting the nut orchard at Agassiz, may be given. Dr. Weir visited Mr. Sharpe at Salmon Arm, B. C., and Mr. Sharpe stated definitely to him that he would be willing to furnish affidavit to the effect that in the main or entirely the chestnut trees in the nut orchard were originally imported from the Orient; in fact, a part of the trees, according to Mr. Sharpe, undoubtedly came from Japan or China and were shipped to Agassiz in the original wrappings, which consisted of the peculiar mats and casings of those countries.

In a letter accompanying the specimens from British Columbia Güssow states that "these trees may be regarded as absolutely isolated. There is no other chestnut tree anywhere round it for 500 miles and more." It seems highly probable therefore that *E. parasitica* was carried to this locality on nursery stock, perhaps as suggested by Faull and Graham and by Weir by importation from the Orient.

The following spring (1913) Mr. Frank N. Meyer, agricultural explorer, discovered this fungus in Chihli Province, China, under such conditions as could leave no doubt that it is indigenous there. The account of this discovery and its corroboration in this country was published by Fairchild (27), and also by the writers (76).

As outlined by Fairchild (27), Meyer first found the diseased chestnuts near Santunying, a small town 1½ days journey by cart from a railroad, northeast of Peking in Chihli Province, between Tsunhuatcho and Yehol.

A small specimen of diseased chestnut bark from this region was inclosed in a letter from Mr. Meyer which was received by Mr. Fairchild on June 28, 1913. From this specimen, which showed only pycnospores, cultures were obtained, which proved it to be true *E. parasitica*. On July 23 more Chinese specimens were received from the same locality, as well as from Scha Ho in the same Province. These included a large canker on a chestnut branch about 6 cm. in

diameter, which agreed in every respect with cankers produced on varieties of Japanese chestnuts in this country (Pl. XXII).

Other specimens in this collection showed well-developed perithecia and ascospores. The ascospore measurements made at the time, as well as the cultures of the Chinese fungus and the inoculation experiments on *C. dentata*, are described in the previous paper by the writers (76, p. 296).

Shortly after this first series of inoculations was made subcultures of the Chinese material were sent to several investigators who had been studying the chestnut-bark disease, in order that the Endothia from China might be tested as soon as possible under American conditions by inoculations at various points throughout the known range of the disease.

A series of inoculations was made by Prof. J. Franklin Collins at Martic Forge, Pa., on July 14, 1913, using American and grafted Paragon and grafted Japanese chestnut trees. Another series of inoculations, 56 in number, was made at the same locality September 10, 1913, by Dr. Caroline Rumbold on grafted Paragon chestnuts. Twenty inoculations were made on native chestnuts at Anderson, Pa., October 2, 1913, by Dr. F. D. Heald and R. A. Studhalter. Inoculations with the Chinese Endothia were made at Leesburg, Va., on both *Castanea dentata* and *C. pumila* by G. Flippo Gravatt and J. T. Rogers, August 16, 1913.

All these investigators made duplicate inoculations with American material, and all agreed that the Chinese fungus was identical in its effects on the host with the American chestnut-blight fungus. During the season of 1914 numerous inoculations with material from China were made by the writers at various points in New Hampshire, Massachusetts, Connecticut, New York, Delaware, and Maryland, while others have been made in Rhode Island by Prof. Collins with the same results.

### ADDITIONAL CHINESE SPECIMENS.

Since the publication of the previous paper (76) additional specimens of *E. parasitica* from China have been received from Meyer; one collected at Changli, Chihli Province, China, October 13, 1913, by Mrs. Mary S. Clemens; a quantity of material collected by Meyer himself in the village of Tachingko, near Taianfu, Shantung, China, March 21, 1914; and another collected by him at Yatyeko, Shensi, China, September 2, 1914. A few cankers have also been sent by Meyer, collected by him at Shihbonshan, near Hangchow, Chekiang Province, China, June 26, 1915. The label on this specimen bears the further comment, "very destructive in this locality." Cultures have been made from all these specimens and have invariably proved to be identical with cultures of *E. parasitica* found in this country.

AN OLD CANKER CAUSED BY ENDOTHIA PARASITICA ON A BRANCH OF CASTANEA MOLLISSIMA.

Collected by Frank N. Meyer, May 31, 1913, near Santunying, Chihli Province, China.

FIG. 1.—JAPANESE CHESTNUT AT NIKKO, JAPAN, FROM WHICH THE CHESTNUT BLIGHT FUNGUS (ENDOTHIA PARASITICA) WAS COLLECTED BY F. N. MEYER, ON SEPTEMBER 17, 1915.

FIG. 2.—TWO BRANCHES OF A JAPANESE CHESTNUT. THE LARGER (TO THE LEFT) WAS BROUGHT TO THIS COUNTRY BY F. N. MEYER, AND FROM IT ENDOTHIA PARASITICA WAS ISOLATED.

As Tachingko is 300 miles south of Changli, where *E. parasitica* was first collected by Meyer, and Yatyeko is 500 miles west of Tachingko, it seems highly probable from the collections that *E. parasitica* is widely distributed in China (fig. 5).

Meyer, writing from Hangchow, July 1, 1915, refers as follows to the condition of the chestnuts in that locality:

Well, I have a few interesting discoveries to report. First, there are many specimens of *Castanea mollissima* scattered at the bases and on the lower slopes of the hills around here, and these chestnuts are seriously attacked by the bark fungus, and in my estimation are going to succumb to it these coming years. The chinquapins (*Castanea* spp.), however, which are very abundant on the higher and more sterile hill slopes, seem to be immune;

FIG. 5.—Outline map of China and Japan, showing the localities in which *Endothia parasitica* has been found.

at least I did not see any evidences of damage or even of attacks. This brings another interesting point to my mind. I was told in Nanking that various missionaries at Kuling, the great summer resort in central China for missionaries, were cutting down their chestnuts, as the tops were all dying, due to borers working underneath the bark.

Meyer has since stated to the writers that he believes the destruction of the chestnut at Kuling is due to *Endothia parasitica* rather than to borers.

In the writers' earlier publication the following statement was made (76, p. 297):

The Chinese organism has thus been shown to be practically identical with the American in all its morphological and physiological characters and in the production of the typical chestnut blight and the pycnidial fructifications

of the fungus. There is apparently but one other requirement that could be made according to the strictest pathological canons to perfect the proof in this case, and that is the production of typical ascospores of *E. parasitica* on the lesions produced by the inoculations.

The last requirement has now been fulfilled. Specimens collected February 15, 1915, from inoculations made September 20, 1913, on chestnuts in Virginia, near Point of Rocks, Md., with Chinese material, show perithecial stromata with typical ascospores of *E. parasitica*, thus completing the evidence.

## DISCOVERY OF ENDOTHIA PARASITICA IN JAPAN.

More than two years after his original discovery of *Endothia parasitica* in China (June 3, 1913), Meyer also discovered the fungus in Japan. A brief account of his discovery has already been published by the writers (78). It may be sufficient here to state that following the discovery of *Endothia parasitica* in China the writers endeavored by correspondence to obtain the fungus from Japan. While not successful in obtaining *Endothia parasitica*, the writers did receive several specimens of fungi, including species of Endothia on species of Castanea. These, together with several specimens of fungi found on chestnut nursery stock from Japan, make it clear that there are in that country several Pyrenomycetes other than *Endothia parasitica* more or less parasitic on Castanea.

Meyer first discovered the chestnut-blight fungus in Japan at Nikko, September 17, 1915, on wild trees of *Castanea crenata* Sieb. and Zucc. A photograph of the trees from which he collected specimens of *Endothia parasitica* is shown in Plate XXIII, figure 1, and a branch from which the diseased material brought to the United Sates was taken is shown in Plate XXIII, figure 2.

Shortly after Meyer's arrival in Washington in December, 1915, the specimens collected at Nikko were turned over to the writers for study. Examination at once showed cankers and mycelial fans typical of *Endothia parasitica*. The material also contained typical pycnospores and ascospores of the fungus. Cultures made from single ascospores on various culture media proved to be identical with those of *Endothia parasitica* found in this country and in China, thus establishing beyond question the identity of the fungus.

Meyer's observations as to the resistance of the Japanese chestnuts to this disease are of great interest. He states that the trees vary considerably as regards their power of resistance, but that in general the Japanese chestnut is even more resistant to *Endothia parasitica* than is the Chinese chestnut (*Castanea mollissima*).

As announced in the same publication (78), *Endothia parasitica* was collected by Dr. Gentaro Yamada at Morioka, northern Japan. These specimens, which show typical cankers as well as ascospores of the fungus, were received by the writers on January 8, 1916.

## PRESENT DISTRIBUTION OF ENDOTHIA PARASITICA IN AMERICA.

The present range of *Endothia parasitica* in America, as shown by the map (fig. 4), is probably merely the extent to which it has been able to spread in the time since it was first introduced.

Whether *Endothia parasitica* was introduced into one locality or several is uncertain, but the studies of Heald (40, 41) and others have shown clearly that the spores of *E. parasitica* are carried by the wind, by insects and birds, and on nursery stock, which would account for its wide distribution and for its occurrence in isolated localities, long distances away from the main body of the disease. It also makes it seem probable that the fungus will continue to spread with some rapidity.

Certainly, there is no evidence that any factor, climatic or otherwise, is likely to prevent the spread of this fungus into the large area of chestnut south of its present range. On the contrary, the duplicate inoculations made by the writers show clearly that the fungus grows more rapidly at the southern limit of its present range than farther north, where it is much more common. The longer growing season in the South is also no doubt an important factor.

In this connection, it may be noted that Köppen (46), in his map of the vegetation regions of the earth, places the portion of China where *Endothia parasitica* has been found indigenous in the same climatic region as that portion of the United States where it is now doing such destructive work. He designates this region as the "Hickory" division of the mesotherms.

## HOST RELATIONS OF THE SPECIES OF ENDOTHIA.

### ENDOTHIA GYROSA.

*Endothia gyrosa* occurs commonly on Liquidambar, Fagus, and Quercus, occasionally on Castanea, and has been found on Vitis in Alabama, but the writers were unable to obtain fresh material from this host.

While Fagus and Quercus are, of course, closely related, it seems remarkable that a fungus should be abundant on hosts so different as Liquidambar and Quercus, yet so rare on any other host as to be only once reported. It seemed possible, indeed, that the fungus on Liquidambar, while morphologically and culturally identical with that on the various other hosts, might prove to be physiologically different. In order to obtain more definite information on this point, several series of cross inoculations were made.

It had been observed that *Endothia gyrosa* was found most frequently on the cut or broken ends of branches or on exposed, bruised, or broken roots. In making inoculations, therefore, a small branch,

1 inch or less in diameter, was cut off about 6 inches from the main trunk. Mycelium from corn meal in flasks was placed on the cut end of the stub and covered with wet cotton, over which oiled paper was tied. In about two weeks the paper and cotton were removed. In all cases, branches similar to those inoculated were cut as checks.

TABLE III.—*Inoculations with Endothia gyrosa.*

| Source of fungus and date. | Host inoculated. | Number of inoculations. | Number successful. | Remarks. |
|---|---|---|---|---|
| Fagus: | | | | |
| May 8, 1913 | Castanea | 6 | 3 | Pycnospores first observed on Oct. 16. |
| May 29, 1913 | Fagus | 3 | 3 | Pycnospores first observed on Aug. 29 for two and on Oct. 10 for the third. |
| Sept. 15, 1913 | Liquidambar | 5 | 2 | No growth until the spring of 1914; pycnidia scattered and small on Oct. 13. |
| Do | Quercus | 4 | 2 | No growth until spring; well developed on Oct. 13, 1914. |
| Apr. 2, 1914 | Fagus | 4 | 0 | |
| Do | Quercus | 4 | 0 | |
| Do | Castanea | 4 | 2 | Pycnidial stromata well developed on Oct. 13, 1914. |
| May 23, 1914 | do | 4 | 4 | Do. |
| Do | Liquidambar | 4 | 0 | |
| Do | Quercus [1] | 4 | 0 | |
| Do | Fagus | 4 | 3 | Do. |
| Quercus: | | | | |
| May 29, 1913 | do | 3 | 3 | Pycnospores first observed on Aug. 29, 1913. |
| Do | Liquidambar | 4 | 2 | Very slight indications of growth on Aug. 29, 1913; a few pycnidia with spores on Oct. 16. |
| Sept. 15, 1913 | do | 8 | 0 | |
| Do | Castanea | 4 | 2 | Large well-developed pycnidia on Oct. 13, 1914. |
| Apr. 2, 1914 | Fagus | 4 | 0 | |
| Do | Quercus [1] | 4 | 0 | |
| Do | Castanea | 4 | 4 | Large abundant pycnidial stromata on Oct. 13, 1914. |
| May 23, 1914 | do | 4 | 4 | Abundant well-developed pycnidial stromata on Oct. 13, 1914. |
| Do | Liquidambar | 4 | 0 | |
| Do | Quercus [1] | 4 | 0 | |
| Do | Fagus | 4 | 3 | |
| Castanea: | | | | |
| May 29, 1913 | do | 3 | 3 | Pycnospores first observed on Aug. 29, 1913. |
| Do | Liquidambar | 3 | 3 | Slight indications of pycnidial formation on Aug. 29, 1913; pycnospores on all on Nov. 17, 1913. |
| Apr. 2, 1914 | Fagus | 4 | 0 | |
| Do | Quercus [1] | 4 | 1 | Large well-developed pycnidial stromata on Oct. 13, 1914. |
| Do | Castanea | 4 | 3 | Scattered, fairly well-developed pycnidia on Oct. 13, 1914. |
| May 23, 1914 | do | 4 | 4 | Abundant well-developed pycnidia on Oct. 13, 1914. |
| Do | Liquidambar | 4 | 0 | |
| Do | Quercus [1] | 4 | 0 | |
| Liquidambar: | | | | |
| May 29, 1913 | Fagus | 3 | 0 | |
| Do | Liquidambar | 3 | 3 | Pycnospores first observed on Aug. 29. |
| Sept. 15, 1913 | Fagus | 8 | 5 | No evidence of growth until the spring of 1914; pycnidia few and small on Oct. 13. |
| Do | Castanea | 6 | 2 | No growth until the spring of 1914; pycnidia small on Oct. 13. |
| Do | Quercus | 6 | 0 | |
| Apr. 2, 1914 | Fagus | 4 | 0 | |
| Do | Quercus [1] | 4 | 0 | |
| Do | Castanea | 4 | 0 | |
| May 23, 1914 | do | 4 | 0 | |
| Do | Liquidambar | 4 | 4 | Abundant pycnidia on Oct. 13, 1914. |
| Do | Quercus [1] | 4 | 0 | |
| Do | Fagus | 4 | 0 | |

[1] The species used in this case was *Quercus prinus*, which proved to be an exceedingly unfavorable host for *Endothia gyrosa*.

Inoculations with *Endothia gyrosa* were also made on numerous hosts from which it had never been reported. Six or more inoculations were made on each host, in the manner described above, except that a part of each series was left unwrapped. The following inoculations showed no growth whatever: Those made in Virginia, April 4, 1914, on *Cornus florida*, *Fraxinus americana*, *Juglans cinerea*, *Ilex opaca*, *Sassafras variifolium;* in Maryland, April 17 and 22, 1914, on *Carya glabra*, *Cornus florida*, *Liriodendron tulipifera*, *Nyssa sylvatica*, *Sassafras variifolium*, and *Quercus alba;* and in New York, July 11, 1914, on *Betula alba*, *Prunus serotina*, *Populus tremuloides*, *Rhus glabra*, *Salix* sp., and *Sassafras variifolium*. On *Acer pennsylvanicum* and Carya two out of the six inoculations developed a few stromata. These were found only on the tissue injured by the cut and there was no evidence of parasitism.

On Castanea, Fagus, Quercus, and Liquidambar, however, a branch inoculated as described above dies back rather faster than the checks. This would indicate, as suggested by Clinton (18, p. 419), that *E. gyrosa* is a weak parasite; that is, that it is able to invade injured and dying tissue.

It is evident from Table III that *Endothia gyrosa* coming from any of the four hosts named will, under favorable circumstances, grow on any of the others. Several other interesting facts are brought out by the table. Inoculations made with material from Liquidambar grew in general more rapidly on Liquidambar than on any of the other hosts. In many cases, material from Liquidambar failed to grow on Castanea, Fagus, and Quercus, and even when inoculations were successful growth was somewhat slower and pycnidial production less abundant.

On the other hand, inoculations from Fagus, Quercus, and Castanea usually grew less rapidly on Liquidambar than on any of the other three hosts. This is, of course, what would be expected from the systematic relationships of the host species, and while the inoculations made are too few to permit any definite conclusions they are nevertheless suggestive. As shown by Table III, *Quercus prinus* proved a very unfavorable host for *Endothia gyrosa*.

In all cases inoculations made in the fall (Sept. 15) failed to show any growth until the following spring. This corresponds with the results in inoculations of *Endothia parasitica*, but it is, of course, impossible to determine whether this failure to grow is due to the dormant condition of the host or to unfavorable weather conditions. Perhaps correlated with the results just noted are the unusually poor results obtained from inoculations made in the early spring. It will be noted that inoculations made on April 2, 1914, were in general much less successful than those made on May 23, 1914, in exactly the same locality and in many cases on the same hosts.

### ENDOTHIA SINGULARIS.

The material of *Endothia singularis* distributed by Sydow as *Calopactis singularis* was on *Quercus gambellii* Nutt. The writers have seen abundant material on this species as well as specimens on *Q. utahensis* (A. DC.) Rydb., *Q. leptophylla* Rydb., and *Q. nitescens* Rydb. Specimens on the latter two hosts were sent by Bethel, who, in a letter, reports finding this species also on *Q. pungens* Liebm.

All of these species except *Quercus leptophylla* are chaparral-forming shrubs growing at an elevation of 4,000 feet or more. There is at present no evidence that the fungus is parasitic on any of the species.

Inoculations with the mycelium of *Endothia singularis* were made on Fagus and on *Quercus alba*, *Q. velutina*, *Q. rubra*, and *Q. palustris*, as well as on *Q. ilicifolia* on Overlook Mountain in the Catskills. No growth has, however, been noted in any case.

### ENDOTHIA FLUENS.

When these investigations were commenced, the writers thought that the Endothia found in Europe might be the same as *Endothia parasitica* found in America. Inoculations were accordingly made in Maryland during October, 1912, with cultures from material collected on the chestnut by the senior writer at Stresa, Italy, and Etrembieres, Switzerland, using material of *E. fluens* sent by P. J. Anderson from Pennsylvania; also material of that species and of *E. parasitica* collected in Virginia as checks. In this case, as in all others where no special mention is made of the method, inoculations were made by cutting through the bark to the wood with a sharp knife. The inoculating material was then inserted with a freshly cut twig and the wound tied up either with cord or rubber bands. If cord was used it was cut away within two to four weeks. The rubber bands became loosened by exposure to the weather within about the same time.

Inoculations were made with all the above material on sprouts of *Castanea dentata* and *Quercus prinus*. The results are summarized in Table IV.

TABLE IV.—*Inoculations of Endothia in Maryland in October, 1912.*

| Fungus. | Host inoculated. | Number of inoculations. | Number showing growth. |
|---|---|---|---|
| Endothia parasitica | Castanea dentata | 32 | 28 |
| Do | Quercus prinus | 6 | 0 |
| E. fluens: | | | |
| European | Castanea dentata | 14 | 14 |
| American | do | 26 | 23 |
| Do | Quercus prinus | 12 | 9 |

The inoculations were examined every 10 days until December 1 and monthly thereafter throughout the winter. There was no perceptible growth until the last of April, when several of the inoculations of *Endothia parasitica* showed slight sunken areas. By May 20 all inoculations checked as showing growth (last column of table) showed the slight yellowish elevations of the bark which indicate the beginnings of pycnidia. On August 30 all the inoculations of *E. parasitica* checked as showing growth had spread rapidly and attacked the living tissues of the host, producing typical cankers with mycelial fans and abundant pycnidia.

No signs of growth were noted in the inoculations of *Endothia fluens* until about the middle of May, 1913, when most of them showed signs of pycnidium formation. By August 30 all those marked as showing growth had produced characteristic pycnidia with spores, which when cultured proved to be typical *E. fluens*. In no case, however, did this fungus spread for any appreciable distance beyond the injured portion or show signs of active parasitism. These results agree with those given by Anderson and Anderson (2, p. 206) with American material of *E. fluens*, and have since been fully confirmed by further observation.

During the summer of 1914 about 1,100 inoculations of *Endothia fluens* from both European and American sources and of *E. fluens mississippiensis* were made on Castanea sprouts. In no case was there any evidence of active parasitism, as in *E. parasitica*.

Although *Endothia fluens* has been found in Europe on a considerable number of deciduous host plants (as recorded on p. 18), the writers have thus far failed to find it in this country on any except Castanea and Quercus. It seemed possible that the European strain of the fungus might be somewhat more plurivorous[1] in its habits than the American. In order to throw some light on this point, the following inoculations were made:

On March 31, 1914, 10 inoculations were made, half of European and half of American material, at Francis, Md., on the following hosts: *Alnus rugosa, Betula nigra, Carpinus caroliniana, Carya glabra, Fagus grandifolia, Liriodendron tulipifera,* and *Liquidambar styraciflua.* Pycnidia appeared only on *Carya glabra* and *Carpinus caroliniana.* Of the inoculations which actually produced pycnidia, four on Carpinus and three on Carya, one of each was the European strain.

On April 22 inoculations were made with American material of *E. fluens* at Kensington, Md., on *Acer rubrum, Carya glabra, Cornus florida, Fagus grandifolia, Prunus serotina, Quercus prinus, Sassafras variifolium, Vaccinium* sp.,

[1] This term is proposed to apply to fungi occurring on two or more hosts or substrata and may be applied to all fungi except true parasites. It is derived from *plus* (plur-), more, and *vorare,* to devour. Compare omnivorous already in use for fungi.

The term pleioxonous might be derived from De Bary's proposed word pleioxony and applied to true parasites having the power to invade more than one species of host plant, and the term plurivorous restricted to nonparasitic organisms.

and *Vitis* sp. Of these, *Acer rubrum* and *Carya glabra* gave numerous small pycnidia.

On July 10 the following hosts were inoculated at Woodstock, N. Y., with *E. fluens* from Europe: *Acer rubrum, A. pennsylvanicum, Carya ovata, Corylus americana, Fraxinus americana, Hamamelis virginiana, Kalmia latifolia, Populus grandidentata, Prunus serotina, Rhus glabra, Salix* sp., *Sassafras variifolium,* and *Syringa vulgaris.* Each host was inoculated in six or seven places, but all failed to develop except two inoculations on *Acer pennsylvanicum* and one on *Corylus americana.*

The results cited above are so largely negative that they prove very little except that the European strain shows no special affinity for these hosts in America.

### ENDOTHIA FLUENS MISSISSIPPIENSIS.

Only five collections of *Endothia fluens mississippiensis* have thus far been made, three on *Castanea dentata* and two on *Quercus* sp. From the results of the inoculations its host relations appear very similar to those of *E. fluens.* The results are shown in Table V.

TABLE V.—*Inoculations with Endothia fluens mississippiensis on Castanea and Quercus.*

| Source of culture. | Host inoculated. | Date. | Number of inoculations. | Number showing pycnidia. |
|---|---|---|---|---|
| Castanea | Castanea | Jan. 20, 1912 | 8 | 8 |
| Do | do | May 8, 1913 | 4 | 4 |
| Do | do | do | 4 | 4 |
| Do | Quercus prinus | do | 9 | 7 |
| Do | Castanea | Apr. 18, 1914 | 12 | 10 |
| Quercus | do | do | 12 | 10 |

The inoculations of January 20, 1912, showed no signs of growth until early in May, when the first signs of pycnidium formation were observed. The inoculations with *Endothia fluens mississippiensis* made May 8, 1913, showed within three weeks discolored areas near the cut which were larger than those about the check cuts. On July 25, 1913, all of the inoculations of *E. fluens mississippiensis* marked "successful" showed the beginnings of pycnidium formation. By August 30, 1913, they were producing pycnospores, which when cultured proved to be *E. fluens mississippiensis.*

Inoculations were made in April, 1914, for the purpose of comparing the material collected on oak with that collected on chestnut. No difference was detected, and there was no indication of active parasitism. This form behaved in this respect exactly as did the *E. fluens* from Virginia both on *Castanea dentata* and *Quercus prinus.*

A series of inoculations parallel to that made with *E. fluens* was made with *E. fluens mississippiensis.* The same hosts were used, and in most cases the dates and places of the inoculation were the same. The results of all that showed any growth are given in Table VI.

TABLE VI.—*Inoculations with Endothia fluens mississippiensis on Acer and Carya.*

| Location. | Host. | Number of inoculations. | Number showing pycnidia. |
|---|---|---|---|
| Woodstock, N. Y. | Acer rubrum | 6 | 3 |
| Do | Carya glabra | 6 | 2 |
| Francis, Md. | do | 6 | 1 |
| Kensington, Md. | Acer rubrum | 1 | 1 |
| Do | Carya glabra | 2 | 2 |

As in *Endothia fluens* the growth was confined to the injured tissues, and there was no evidence of parasitism.

### ENDOTHIA TROPICALIS.

The material of *Endothia tropicalis* from which the writers secured their cultures, was collected by T. Petch in Ceylon. As the species of Endothia in the Northern Hemisphere are chiefly on members of the Fagaceæ, Petch's statements with regard to hosts are of considerable interest. In a letter of March 6, 1914, he writes:

> We have no Fagaceæ native in the island. We have introduced various species of *Quercus* and *Castanea*, but subsequent to Thwaite's discovery of this fungus. I do not think there can be any doubt that the fungus is native to Ceylon * * *
>
> Of the speciments now sent * * * those in the packet * * * are from a tree which was producing shoots from the base. This tree is *Elaeocarpus glandulifer* Mast. From the bark and habit, I believe that all my "finds" of *Endothia* have been on this species.
>
> In the accounts of the American chestnut disease, I notice that several authors speak of "cankers," and give their rate of growth. I never see "cankers" (Krebs) on the Ceylon trees. The bark appears to die regularly and smoothly from above downward, and is quite unbroken except for the minute cracks through which the stromata emerge.

*Inoculations.*—As already noted, ascospores of *Endothia tropicalis* resemble those of *E. parasitica* even more closely than do those of *E. fluens*. This fact, together with its similarity on culture media and its oriental origin, led the writers to fear possible parasitic tendencies.

Inoculation experiments were accordingly made only on the chestnut and under carefully guarded conditions. In all, about 30 inoculations were made on 2-inch chestnut sprouts, using the methods described for other species.

Of 25 inoculations made in May and June, practically all had developed a few pycnidial stromata by October 20. These stromata were a somewhat brighter orange than those of *E. fluens* or *E. fluens mississippiensis*, and the spores when cultured produced typical *E. tropicalis*. In no case, however, was there any evidence of parasitism.

### ENDOTHIA PARASITICA ON HOSTS OTHER THAN CASTANEA.

The first collection of *Endothia parasitica* on a host other than Castanea of which the writers have any knowledge is that made by J. Franklin Collins at Martic Forge, Pa., June 30, 1909. As announced by Dr. Metcalf at the Boston (December, 1909) meeting of the American Phytopathological Society, the specimen consisted of a small dead branch of *Quercus velutina* with several spore tendrils typical of *E. parasitica*. This material, which consisted of a terminal branch with leaves still retained, was at once sent to the laboratory at Washington, and cultures obtained from it were subsequently used in making numerous inoculations on *Castanea dentata* on Long Island, N. Y., in July, 1909. On November 17 of the same year, Metcalf reported that the inoculations were entirely successful and had produced typical lesions, thus establishing without question the identity of the fungus.

Fulton (37, p. 53) reports *E. parasitica* on the dead bark of *Quercus alba* and *Quercus velutina*, but found no evidence that the fungus produces in any sense a disease of such trees. Clinton (18, p. 428) mentions cultures from three different species of Quercus and (p. 376) reports specimens on *Quercus alba*, *Q. rubra*, and *Q. velutina*.

Anderson and Babcock, as quoted by Anderson and Rankin (6, p. 564), found *Endothia parasitica* on *Quercus velutina*, *Q. alba*, *Q. prinus*, *Rhus typhina*, *Acer rubrum*, and *Carya ovata*, but it seemed parasitic only on *Quercus alba*. They made inoculations with materials isolated from Castanea on *Quercus prinus*, *Q. velutina*, *Q. alba*, *Q. coccinea*, *Rhus typhina*, *Acer rubrum*, *Liriodendron tulipifera*, and *Carya ovata*. Two trees of Rhus were girdled and killed by the growth of the fungus. On *Quercus alba* the fungus seemed slightly parasitic, but none of the trees were killed. The fungus grew and produced spore horns on the wounded tissue near the point of inoculation on all the hosts except Acer and Liriodendron.

Rankin (62, p. 238) also made inoculations with *Endothia parasitica* from Castanea on *Quercus prinus*, *Q. rubra*, *Q. alba*, *and Q. coccinea*. He found that the mycelium advanced into the living tissues for a short distance in a few cases, but that in no case were typical cankers formed. Pycnidia were produced abundantly on the injured tissues of all the hosts.

During the course of this work only four specimens of *Endothia parasitica* on hosts other than Castanea have come to the writers. One was on chestnut oak (*Quercus prinus*) collected by F. W. Besley, at Towson, Md., December 26, 1911; one from *Quercus velutina*, at Germantown, Pa., as well as one from white oak (*Quercus alba*), at Kennett Square, Pa., were collected by S. B. Detwiler; and one from dead maple, *Acer* sp., at Florence, Mass., by Roy G. Pierce.

The specimen collected by Besley on *Quercus prinus* showed the fan-shaped mats of mycelium typical of *E. parasitica* on Castanea species. The fungus had apparently girdled the tree. The specimen on *Quercus alba*, collected by Detwiler, was similar to one on *Quercus prinus* in appearance and came from a dead tree which had apparently been killed by the growth of the fungus. The specimens on *Acer* sp. and on *Quercus alba* were received in the spring of 1914, and cultures isolated from them were used in making inoculations for the purpose of determining whether the fungus had either lost or gained in virulence by passing through other hosts.

### INOCULATION EXPERIMENTS.

The cultures secured from Acer and Quercus, together with one made from Castanea at about the same time, were inoculated into three separate sprouts of *Acer rubrum*, *Castanea dentata*, and *Quercus prinus*. The sprouts chosen were of nearly the same size, 2 inches in diameter, and similarly situated, and each was inoculated in five places, with two check cuts above. The inoculations were made the usual way on March 31, 1914, and were examined at least once a month during the summer.

None of the inoculations on Quercus produced any growth whatever. On Acer the inoculations with the culture from Quercus all failed to develop; one of the inoculations with the culture from Acer showed a few pycnidia, while four of the inoculations with material from the chestnut developed a few pycnidia. On Castanea the three series of inoculations were almost identical, every inoculation producing a typical canker.

Of course, these inoculations are too few to be conclusive, but it is evident that there was no decrease in virulence on the chestnut in passing through Acer or Quercus and that no particular affinity for either Acer or Quercus was gained. On the maple, in fact, the culture direct from chestnut produced the most growth.

In addition to those listed above, numerous inoculations were made in order to determine whether *Endothia parasitica* had any parasitic tendencies on other deciduous hosts.

These inoculations were all made during the spring of 1914 by the usual method of cutting well through the bark and inserting mycelium and spores from a pure culture, usually on corn meal. The wounds were then wet, some bound with wet cotton, others with paraffin paper, and about half were left unwrapped.

Seven or more inoculations were made on April 4 in Maryland on *Alnus rugosa*, *Betula nigra*, *Carpinus caroliniana*, *Fagus grandifolia*, *Kalmia latifolia*, *Liriodendron tulipifera*, and *Liquidambar styraciflua*, none of which developed. Inoculations were also made on April

22 in this locality on *Acer rubrum*, *Carya glabra*, *Cornus florida*, *Fagus grandifolia*, *Liriodendron tulipifera*, *Quercus prinus*, *Sassafras variifolium*, *Vaccinium* sp., and *Vitis* sp. without success. On April 18, the following hosts were inoculated in Virginia: *Acer rubrum*, *Betula nigra*, *Benzoin aestivale*, *Carpinus caroliniana*, *Carya glabra*, *Cornus florida*, *Fagus grandifolia*, *Liriodendron tulipifera*, *Prunus serotina*, *Quercus alba*, *Ulmus americana*, and *Vitis* sp. Each host was inoculated in from four to six places. Of these, pycnidia were produced only on *Acer rubrum*, *Carpinus*, and *Liriodendron*. A similar series was made on the same hosts in the same place on May 27. Inoculations on one tree of *Quercus alba* showed undoubted evidence of parasitism and is described below.

On July 9 and 11 from five to fourteen inoculations were made on each of the following hosts at Woodstock, N. Y.: *Acer rubrum*, *Betula alba*, *Carya ovata*, *Fagus grandifolia*, *Fraxinus americana*, *Hamamelis virginiana*, *Juglans cinerea*, *Kalmia latifolia*, *Nyssa sylvatica*, *Ostrya virginiana*, *Populus grandidentata*, *Prunus serotina*, *Rhus typhina*, *Quercus rubra*, *Salix* sp., *Sambucus canadensis*, and *Sassafras variifolium*. Pycnidia appeared on *Acer rubrum* and Ostrya only. The fungus made considerable growth on two plants of *Rhus typhina*, partly girdling branches one-half inch in diameter and producing distinct fans. The fans were, however, much smaller than those usually found in Castanea. Inoculations were made at Avon, Conn., July 15, on *Acer saccharum*, *Betula alba*, *Carya glabra*, *Cornus florida*, and *Ostrya virginiana*. Pycnidia developed only on Ostrya. The successful inoculations with *Endothia parasitica* are shown in Table VII.

TABLE VII.—*Successful inoculations in 1914 with Endothia parasitica on hosts other than Castanea.*

| Locality. | Date. | Host. | Number of inoculations. | Number successful.[1] |
|---|---|---|---|---|
| Virginia | Apr. 18 | Acer rubrum | 9 | 7 |
| Do | do | Carpinus caroliniana | 6 | 2 |
| Do | do | Liriodendron tulipifera | 6 | 1 |
| Do | May 27 | Quercus alba | 4 | 4 |
| New York | July 11 | Acer pennsylvanicum | 14 | 4 |
| Do | do | Ostrya virginiana | 6 | 2 |
| Connecticut | July 15 | do | 15 | 4 |

[1] Inoculations producing pycnidia are classed as successful.

It must be noted that while pycnidia were produced in the cases listed as successful, there was no indication of parasitism, nor did the growth extend beyond the tissue injured by the cut except in Quercus and Rhus.

Out of about 400 inoculations with *Endothia parasitica* on hosts other than Castanea, about 70 of which were made on different

species of Quercus, chiefly *Q. prinus* and *Q. alba*, only one case has been noted in which the fungus assumed a typically parasitic rôle. The data in this case may be summed up as follows: Four inoculations were made May 27, 1914, on a small tree of *Quercus alba*. This tree was suppressed, and although when cut down it showed about 30 annual rings it was only 16 feet high and about 2 inches in diameter. It was in a moist, shady locality close beside a stream, and in spite of its small size was apparently healthy. The inoculations were made in the usual way from a culture of *E. parasitica* on corn meal. On August 1 it was noted that all four inoculations were producing pycnidia, and in at least one case typical fans had been developed. On October 15 all four cankers had more than half girdled the seedling. No observations were made during the winter, but at the time the leaves had reached half the normal size, in the spring of 1915, the tree was completely girdled. On July 1 this tree presented an appearance closely similar to that of a small chestnut tree girdled by *Endothia parasitica*. All the leaves above the point of inoculation were dead and remained attached to the branches. Below the girdled portion, water sprouts had developed, as has been frequently described for chestnut trees affected with *E. parasitica*. Cultures made from this tree showed the fungus to be typical of *E. parasitica*. Whether this case of parasitism was due to unusual virulence on the part of the fungus or to unusual susceptibility on the part of the host is, of course, merely a matter of conjecture; the latter alternative seems, however, somewhat more probable, as other inoculations with this strain of the fungus on *Q. prinus* and *Q. alba* failed to show similar results.

In addition to the above, a somewhat similar observation has been made by the writers near Amherst, Mass. In connection with other work, a sprout of *Quercus prinus* about an inch in diameter was inoculated with *Endothia gyrosa* on July 15, 1914. When this inoculation was made the tree was partly (about one-fourth) girdled. *E. gyrosa* developed normally and by October 1, 1914, had produced several pycnidial stromata. No change was apparent when the inoculations were examined in May, 1915.

*E. parasitica* was abundant in the region, however, and apparently gained entrance through the cuts originally made, for when the plat was next visited, August 17, 1915, the sprout appeared quite dead, though still retaining its full-sized dead leaves. Further examination showed numerous pycnidia of *E. parasitica* in addition to those of *E. gyrosa* near the region of the original inoculation. The pycnidia of *E. parasitica* were on all sides of the stem, while those of *E. gyrosa* were confined to the portion above the cuts made in inoculating. The mycelial fans typical of *E. parasitica* were abundant also. These

observations leave no doubt that the tree was girdled and killed by *E. parasitica.*

*Endothia parasitica* in exceptional cases undoubtedly attacks other hosts than Castanea, producing cankers and sometimes causing the death of the host. The results of the inoculations just recorded appear to indicate that some unusual conditions of host or parasite must obtain in such cases. Whether such a combination of conditions or factors will ever become sufficiently frequent to lead to serious destruction of Quercus or other forest trees remains to be determined.

### ENDOTHIA PARASITICA ON CASTANEA SPP.

Although found occasionally on species of other genera, *Endothia parasitica* is dangerously pathogenic only on members of the genus Castanea. The parasitism of this fungus on the American chestnut (*Castanea dentata*) was first proved by Murrill (57) and has since been demonstrated by numerous investigators.

When *Endothia parasitica* was discovered in the United States it was considered by some investigators to be a native fungus which had suddenly become parasitic, and various theories were advanced to account for the supposed unusual susceptibility of the host. As enumerated by Clinton (18, p. 391), the factors suggested include winter injury, drought injury, fire injury, weakened condition due to continued coppicing, and reduced amounts of tannic acid due perhaps to weather conditions.

Continued study by many investigators in different localities has, however, fully confirmed the observation originally made by Metcalf and Collins in 1910 (53) that "a debilitated tree is no more subject to attack than a healthy one" and that *Endothia parasitica* is actively parasitic on the healthiest specimen of *Castanea dentata* in case there is opportunity for wound infection. The writers have personally made over 1,200 inoculations of *E. parasitica* on *Castanea dentata* without finding a single individual that showed any resistance.

### CASTANEA ON LIMESTONE SOILS.

Not only are all trees susceptible, but so far as is known no condition of soil, altitude, or moisture renders them more resistant to the disease. The idea has been held by some writers that chestnuts grown on limestone soils were immune to the disease, and the planting of chestnut orchards on such soils was advocated. This view is held by Gulliver (38, p. 53), who sums up his observations in two regions in Pennsylvania as follows:

> In every series of tracts taken from limestone to overlying shale soils, the percentage of blight is least at a comparatively short distance * * * from the edge of the limestone. Tracts on soils derived from limestone which show the highest percentage of blight seem to be those where the soil has

become acid from underground drainage. Chestnut trees on soils derived from other alkaline rocks show less blight than is found in the trees on shale soils with limestone underneath.

On the other hand, Detwiler (24, p. 67) reports observations in the Lizard Creek valley which seem to show that these relations do not always occur. He says—

> A belt of limestone borders Lizard Creek valley on the south, and the per cent of infection is as high in that region as elsewhere. Infection centers have been found near limestone quarries, where the roots of the chestnut penetrated to bedrock.

Actual proof or disproof of the truth of this idea was peculiarly difficult, since chestnut is but rarely found growing naturally on calcareous soils. During the summer of 1914, however, a careful study of the chestnut on certain portions of limestone areas in western Maryland and western Connecticut was made. These localities were chosen because they were convenient in connection with other work, the blight had been present for several years in both States, and thorough State geological surveys made the location of the limestone areas very easy. The two States also are sufficiently far apart to eliminate sources of error that might arise from local weather conditions.

In western Connecticut chestnut was abundant on glacial till over the Stockbridge limestone of this region. Chestnut was also growing directly over limestone at various points near Danbury, Twin Lakes, Chapinville, and Lakeville. Several localities near the latter place were kindly pointed out by Dr. George E. Nichols. Near Danbury every tree examined showed the blight in a more or less advanced stage, while near the other towns, all in the northwest corner of the State, nearly 50 per cent of the trees were blighted. About 30 inoculations were made on sprouts in this region, and all except two developed cankers quite as rapidly as did check inoculations made on the trap ridge west of Hartford.

Chestnut is very rare on the Shenandoah limestone in the Hagerstown and Frederick valleys of western Maryland. A number of chestnut trees were, however, located growing on limestone soil near Frederick Junction and Adamstown in the Frederick valley. The disease was already established west of Adamstown, where 20 per cent of the chestnuts were either diseased or dead. Twenty-two inoculations were made on nine chestnut sprouts in these two regions, and all developed typical cankers quite as rapidly as the checks made in similar sprouts growing over Baltimore gneiss 50 miles east.

### RECESSION OF THE CHESTNUT IN THE SOUTHERN STATES.

While it has been definitely proved that *Endothia parasitica* is pathogenic on healthy chestnut trees, one of the points brought for-

ward by the advocates of the "weakened host" theory seems to be fully established; that is, that the chestnut trees have suffered severely in the southern Appalachian regions previous to the present epidemic, in some cases being practically exterminated, so that the range is now considerably less than formerly. The evidence on this point has been summarized by Clinton (18, pp. 408–413). Various writers quoted by him cite fire injury and borers and other insects as causes for this recession.

Long (48, p. 8) considers a root rot due to *Armillaria mellea* as "very probably an important factor in the gradual recession of the chestnut" in North Carolina. It seems probable that all of the above-mentioned factors, and perhaps others, have played a part in the destruction of the chestnut in this region.

### RELATIVE SUSCEPTIBILITY OF SPECIES OF CASTANEA.

The importance of *Castanea dentata* as a timber and nut tree and its abundance in eastern North America, where the blight is prevalent, has made the chestnut blight an object of much investigation. Descriptions of the nature and importance of the disease, the rate of its spread, methods of distribution, and attempted methods of control have been given in detail by Anderson (1–5), Clinton (12–15), Heald (39–41), Metcalf (51 and 52), Metcalf and Collins (53), Rankin (62), and others. It may be sufficient here to state that the fungus enters the host through a wound in the bark, probably never or very rarely through lenticels or natural cracks, grows chiefly in the cambium, penetrating for only short distances into the wood, and kills the tree or branch by girdling. Once a tree is attacked, it is only a question of time till it succumbs.

The chinquapin (*Castanea pumila*) was found by Murrill (58) in 1908 to be attacked by *Endothia parasitica*. Rogers and Gravatt (65) in 1915 made inoculations of *E. parasitica* on *C. pumila* and found that the parasite grew as rapidly on this host as on *C. dentata*. They attribute the apparent resistance of the chinquapin to its comparative freedom from bark injury, a view also held by other writers. Pantanelli (60) and Metcalf (52) have proved that the European chestnut is readily susceptible to the disease.

The only chestnuts thus far observed which show any resistance to *Endothia parasitica* are those of oriental origin. Metcalf (51) first pointed out the resistance of the Japanese chestnut. This observation has since been confirmed by Clinton (18, p. 375), who "failed to produce the disease in a Japanese variety in the [Conn.] station yard, although the bark was inoculated in 16 different places."

Van Fleet (84), in describing the spread of the chestnut blight in his breeding plats at Washington, D. C., says (p. 21): "The Asiatic chestnuts and the chinquapin-Asiatic hybrids are plainly highly resistant."

Morris (56) sums up eight years' observation of the effect of the chestnut blight on 26 species and varieties of chestnuts at Stamford, Conn., as follows:

> Every one of the 5,000 American chestnut trees became blighted * * * None of [the grafted varieties or seedlings of European and Asiatic varieties appear] to be as vulnerable as the American chestnut, but most of mine are now dead. Korean chestnuts and chestnuts from the Aomori regions in Japan resisted the blight until six years of age. Since that time they have shown a marked tendency to blight, but resist it better than does the American chestnut * * * None of the American species of chinquapin * * * has blighted with the exception of two limbs * * * None of the specimens of *Castanea alnifolia* [or] * * * of *Castanea mollissima* has blighted, but these latter include only five trees.

These observations as to the resistance of the oriental varieties of chestnut when grown in America are of particular interest in connection with the observations of Meyer in the region where he discovered *Endothia parasitica* native. In his letter to Fairchild, written from Santunying, China, June 4, 1913, Meyer makes the following notes with reference to the effect of the blight in that region:

> This blight does not by far do as much damage to Chinese chestnut trees as to the American ones * * *
>
> Not a single tree could be found which had been killed entirely by this disease, although there might have been such trees which had been removed by the ever-active and economic Chinese farmers * * *
>
> Dead limbs, however, were often seen and many a saw wound showed where limbs had been removed * * *
>
> The wounds on the majority of the trees were in the process of healing over * * *
>
> Old wounds are to be observed here and there on ancient trees.

Meyer's photographs taken near Santunying substantiate his statements. Certainly no specimens of *C. dentata* in a blight-infested region in this country could survive to the age of the Chinese chestnuts shown in his photographs.

That the Chinese chestnuts are by no means uniformly resistant, however, is clearly shown by Meyer's later notes. On the label of a package of *Endothia parasitica* collected on chestnut at Tachingko, Shantung, China, March 21, 1914, he writes, "Trees very severely attacked, many dying off," and in a letter written from the same place he says, "A serious canker; many of the trees here were killed by it."

Further evidence that the virulence of *Endothia parasitica* on Chinese chestnut differs in different parts of China is found in subsequent communications from Meyer. From a point near Chingtsai, Chekiang, China, on July 15, 1915, he writes: "All around Hangchow and west of it one finds the chestnut trees seriously attacked by this destructive bark fungus."

On July 11, 1915, near Changhua, Chekiang, China, he comments, "With the exception of near Taianfu, Shantung, chestnuts

are much more severely attacked in the Chekiang Province than either in Chihli, Shansi, or Shensi. Could the greater humidity of central China be of assistance to a more vigorous development of this destructive fungus?"

### COMPARISON OF HOST RELATIONS.

It will be seen from the above description of the host relations of the various species that while some other members of the genus (*E. gyrosa*, e. g.) may have slight parasitic tendencies, *Endothia parasitica* alone is an active parasite. The contrast is still more striking in the section of the genus to which *E. parasitica* belongs, for *E. fluens* and *E. fluens mississippiensis*, which resemble *E. parasitica* so closely in their morphological characters, and to a less degree on culture media, and are common on Castanea, are almost purely saprophytic. This fact is established by the work of Anderson (2), Clinton (18), and others, and by two years' field observations and several thousand inoculations made by the writers and their colleagues.

The host relations of the parasite are equally striking. Although *Endothia parasitica* is so pathogenic on *Castanea dentata* that this tree has been practically exterminated over several hundred square miles of its natural range and its extinction is threatened, the fungus has been only occasionally found as even a weak parasite on the closely related genus Quercus, and never, to the writers' knowledge, on Fagus.

During the course of this work the writers have been continually impressed with the possibilities of a physiological study of *E. parasitica* and one or more closely related species which might throw some light on the fundamental question of the nature and cause of parasitism. No other case is known to the writers of a virulently parasitic fungus and a closely related purely saprophytic one which will grow readily and fruit on a large variety of artificial media, which are readily distinguishable on those media, and remain constant for hundreds of generations.

## SUMMARY.

The pathological and economic importance of this group of fungi was first recognized when the chestnut-blight fungus was discovered in New York in 1904.

This organism was first referred to the genus Diaporthe, but was later shown to belong to the genus Endothia.

The specific identity, relationships, and native home of this parasite were at first uncertain. Some pathologists considered it a native organism which was attracting attention and causing injury chiefly

by reason of the weakened condition of the chestnut trees. Others believed it to be of foreign origin. Its recent discovery in China and Japan has settled this question.

To determine positively the identity of the organism, a thorough study was made of the types or authentic specimens of all the species of Endothia obtainable. As a result of this work a revision of available species of the genus is presented. This is based upon the field and laboratory study of over 600 collections. Over 4,000 cultures have also been studied.

*Endothia gyrosa* (Schw.) Fr. is the type of the genus, which is naturally divided into two sections, chiefly by the character of the ascospores. In section 1 they are short, cylindric to allantoid, and continuous or only pseudoseptate. This section contains two species, *E. gyrosa* and *E. singularis*.

Section 2 has oblong-fusiform to oblong-ellipsoid uniseptate ascospores. This contains four species and one variety, *Endothia fluens*, *E. fluens mississippiensis*, *E. longirostris*, *E. tropicalis*, and *E. parasitica*. *E. tropicalis* is a hitherto unrecognized species.

Radiating layers of yellowish or buff mycelium situated in the bark and cambium of the host are found to be constant and distinctive characteristics of *Endothia parasitica*. None of the other species studied shows this character.

All species of the genus possess a stroma having a distinctive yellow to reddish color.

There is no division of stroma into distinct layers, as described by some authors. Pycnidia or perithecia may arise in any portion of the stroma. Most commonly where pycnidia and perithecia are both present the pycnidia are above the perithecia, though the reverse arrangement is sometimes observed and all intermediate conditions frequently occur.

The stromata of the species of section 1 are larger, more erumpent, and contain more numerous pycnidia than those of section 2. *Endothia singularis* is especially striking in this respect. The stromata of section 2 are smaller and very similar in all the species.

The pycnidia consist of more or less irregular chambers or locules in the stroma.

The pycnospores are small in most species and furnish no very distinctive specific characters. The pycnospores of *Endothia tropicalis* are, however, constantly larger and more variable in size than those of the other species.

Paraphyses have been described by some authors, but have never been observed by the writers.

The ascospores in the species of section 1 are very similar in size and shape. Those in section 2, though similar, have been found by thorough study and careful measurement to show constant though

slight differences, as indicated in the tables of measurements and ratios.

Numerous cultures of all the species on a variety of media show that each species has constant and distinctive characters of growth and color.

All the species grew equally well in light or darkness, and no decided differences in temperature relations have been demonstrated.

The species appear to have well-defined geographic limits of distribution, which have been approximately determined for the American species. The distribution of the species does not coincide with that of the hosts, but seems to be determined in part by soil and climatic conditions.

*Endothia fluens* has the widest distribution, being frequent and widely distributed in Europe and the eastern United States, and also occurring in Asia.

*Endothia parasitica* is evidently of oriental origin. Specimens have been received from five rather widely separated localities in China and from two localities in Japan. In the eastern United States it is now abundant from Maine to North Carolina and is rapidly spreading south and west. It has already destroyed most of the chestnut trees within a radius of 100 miles of New York City.

The species have rather definite host relations.

*Endothia gyrosa* has been found on five genera of plants, viz, Castanea, Fagus, Liquidambar, Quercus, and Vitis.

*Endothia singularis* occurs, so far as known, only on Quercus species.

*Endothia fluens* has been found in America only on Castanea and Quercus, but in Europe it occurs on Alnus, Carpinus, Castanea, Corylus, Quercus, and Ulmus, and has been reported on Aesculus, Fagus, and Juglans.

*Endothia fluens mississippiensis* has been found only on Castanea and Quercus.

*Endothia tropicalis* is known only on Elaeocarpus.

*Endothia parasitica* has been found on Acer, Carya, Castanea, Quercus, and Rhus, but at present is only known as a serious parasite on Castanea.

Upon the American species of Castanea it is actively parasitic under all the conditions of soil and climate observed. Oriental species of chestnut are more or less resistant to the disease both in America and their native homes.

None of the species except *Endothia parasitica* has thus far been found to be actively parasitic.

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